#	1 mM GSH	0.1 mM GSSG	0.3 mM LM	[illegible] mM NaCl	[illegible] mM KCl	0.055% PEG 3350	550 mM GndHCl	1.1 mM EDTA	2.2 mM $MgCl_2$	2.2 mM $CaCl_2$	440 mM Sucrose	550 mM L-Arg	Activity U/g IB
2 (55 mM MES pH 6.5)	+	+	+	10.56	0.44	0	+	0	+	+	0	0	0
3 (55 mM MES pH 6.5)	+	+	0	10.56	0.44	+	+	+	0	0	+	+	0
#5 (55 mM MES pH 6.5)	+	+	0	264	11	0	0	0	+	+	+	0	0
#8 (55 mM MES pH 6.5)	+	+	+	264	11	+	0	+	0	0	0	+	40.00
#10 (55 mM Tris pH 8.2)	+	+	+	10.56	0.44	0	0	+	0	0	+	0	0
#11 (55 mM Tris pH 8.2)	+	+	0	10.56	0.44	+	0	0	+	+	0	+	105.26
#13 (55 mM Tris pH 8.2)	+	+	0	264	11	0	+	+	0	0	0	0	15.65
#16 (55 mM Tris pH 8.2)	+	+	+	264	11	+	+	0	+	+	+	+	48.70

Figure 1

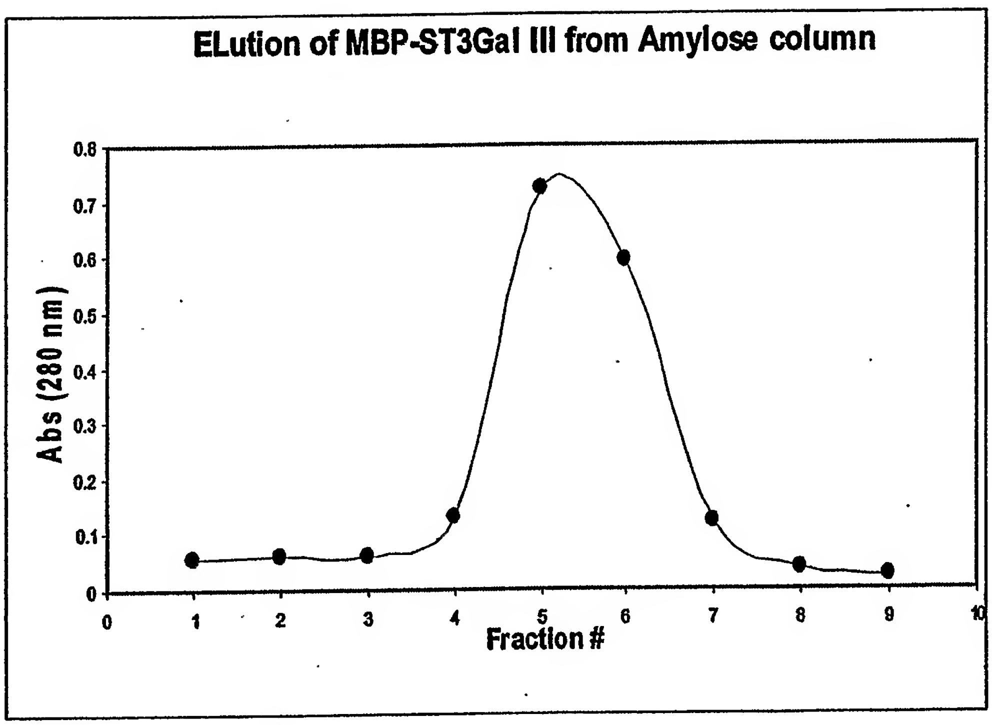

ELution of MBP-ST3Gal III from Amylose column
Abs (280 nm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
Fraction #

Figure 2

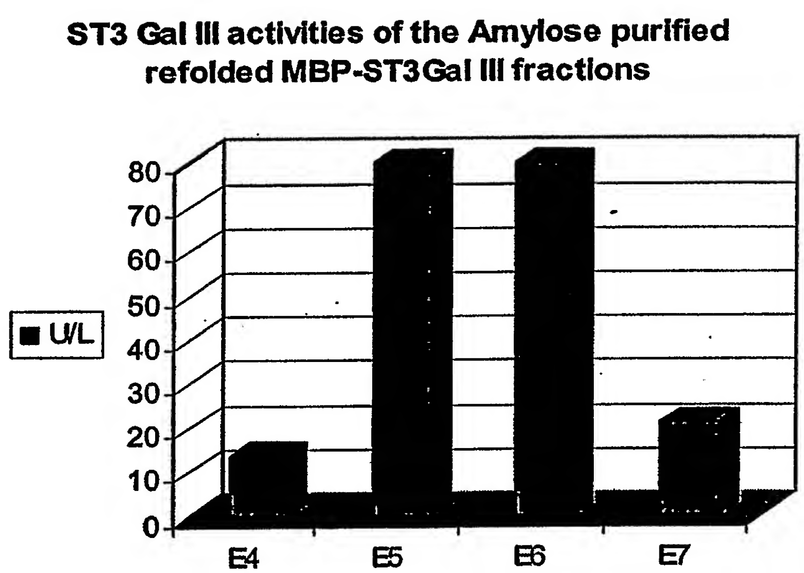

ST3 Gal III activities of the Amylose purified
refolded MBP-ST3Gal III fractions
80
70
60
50
40
30
20
10
0
U/L
E4
E5
E6
E7

Figure 3

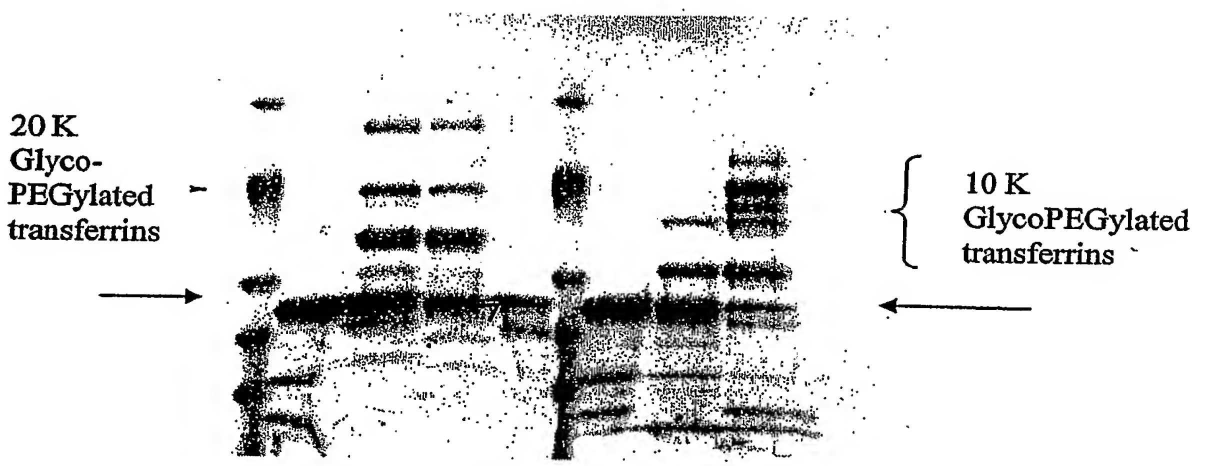

20 K
Glyco-
PEGylated
transferrins
10 K
GlycoPEGylated
transferrins

Figure 4

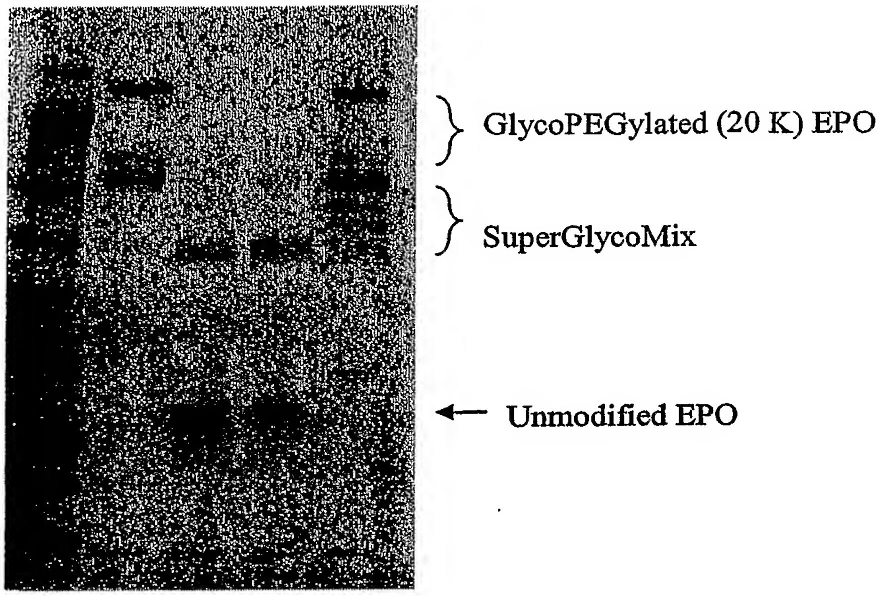


Figure 5. GlycoPEGylation (20 K)of EPO

Figure 5

```
               10        20        30        40        50        60
/usr/t MLKKQSAGLVLWGAILFVAWNALLLLFFWTRPAPGRPPSVSALDGDPASLTREVIRLAQD
       ::::::::::::::::::::::::::::::::.:.: :: .::: :::::::::::::::
P27115 MLKKQSAGLVLWGAILFVAWNALLLLFFWTRPVPSRLPSDNALDDDPASLTREVIRLAQD
               10        20        30        40        50        60

               70          80        90       100       110
/usr/t AEVELERQRGLLQQIGD--ALSSQRGRVPTAAPPAQPRVPVTPAPAVIPILVIACDRSTV
       ::::::::::::::: .   :: ::: .::::::::::.::::: ::::::::::::::::
P27115 AEVELERQRGLLQQIREHHALWSQRWKVPTAAPPAQPHVPVTPPPAVIPILVIACDRSTV
               70        80        90       100       110       120

      120       130       140       150       160       170
/usr/t RRCLDKLLHYRPSAELFPIIVSQDCGHEETAQAIASYGSAVTHIRQPDLSSIAVPPDHRK
       ::::::::::::::::::::::::::::::::.:::::::::::::::::.::: :::::
P27115 RRCLDKLLHYRPSAELFPIIVSQDCGHEETAQVIASYGSAVTHIRQPDLSNIAVQPDHRK
              130       140       150       160       170       180

      180       190       200       210       220       230
/usr/t FQGYYKIARHYRWALGQVFRQFRFPAAVVVEDDLEVAPDFFEYFRATYPLLKADPSLWCV
       :::::::::::::::::.:..: .::::::::::::::::::::.:::::::::::::::
P27115 FQGYYKIARHYRWALGQIFHNFNYPAAVVVEDDLEVAPDFFEYFQATYPLLKADPSLWCV
              190       200       210       220       230       240

      240       250       260       270       280       290
/usr/t SAWNDNGKEQMVDASRPELLYRTDFFPGLGWLLLAELWAELEPKWPKAFWDDWMRRPEQR
       :::::::::::::.:.::::::::::::::::::::::::::::::::::::::::::::
P27115 SAWNDNGKEQMVDSSKPELLYRTDFFPGLGWLLLAELWAELEPKWPKAFWDDWMRRPEQR
              250       260       270       280       290       300

      300       310       320       330       340       350
/usr/t QGRACIRPEISRTMTFGRKGVSHGQFFDQHLKFIKLNQQFVHFTQLDLSYLQREAYDRDF
       .::::.::::::::::::::::::::::::::::::::::: ::::::::::.:::::::
P27115 KGRACVRPEISRTMTFGRKGVSHGQFFDQHLKFIKLNQQFVPFTQLDLSYLQQEAYDRDF
              310       320       330       340       350       360

      360       370       380       390       400       410
/usr/t LARVYGAPQLQVEKVRTNDRKELGEVRVQYTGRDSFKAFAKALGVMDDLKSGVPRAGYRG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
P27115 LARVYGAPQLQVEKVRTNDRKELGEVRVQYTGRDSFKAFAKALGVMDDLKSGVPRAGYRG
              370       380       390       400       410       420

      420       430       440
/usr/t IVTFQFRGRRVHLAPPPTWEGYDPSWN
       :::: ::::::::::: ::.::::::.
P27115 IVTFLFRGRRVHLAPPQTWDGYDPSWT
              430       440
```

Figure 6

GnT1 Cys121Ser mutant

avipilviacdrstvrrsldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa
lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg
apqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn*

Gcggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcggcgctctctagacaagctgctgcattatcggccctcggctga
gctcttccccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcaca
tccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactaccg
ctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggaggtggccccggacttctt
cgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagca
gatggtggacgccagcaggcctgagctgctctaccgcaccgactttttccctggcctgggctggctgctgttggccgagctctgggct
gagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcagggggcgggcctgcatacg
ccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctga
accagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacggt
gctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt
ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggagggctatgatcctagctggaattag

Figure 7

GnT1 Cys121Asp

avipilviacdrstvrr**d**ldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa
lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg
apqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn*

Gcggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcggcgc**gat**ctagacaagctgctgcattatcggccctcggctg
agctcttccccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcac
atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc
gctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggaggtggccccggacttct
tcgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagc
agatggtggacgccagcaggcctgagctgctctaccgcaccgactttttccctggcctgggctggctgctgttggccgagctctgggc
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcaggggcgggcctgcatac
gccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctg
aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacgg
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt
ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggagggctatgatcctagctggaattag

Figure 8

GnT1 Cys121Thr

avipilviacdrstvrrtldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwal
gqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwael
epkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg
apqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn*

Gcggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcggcgcactctagacaagctgctgcattatcggccctcggctg
agctcttccccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcac
atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc
gctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggaggtggccccggacttct
tcgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagc
agatggtggacgccagcaggcctgagctgctctaccgcaccgactttttccctggcctgggctggctgctgttggccgagctctgggc
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcaggggcgggcctgcatac
gccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctg
aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacgg
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt
ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggagggctatgatcctagctggaattag

Figure 9

GnT1 Cys121Ala

avipilviacdrstvrraldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrwa
lgqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwae
lepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvyg
apqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn*

Gcggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcggcgcgccctagacaagctgctgcattatcggccctcggctg
agctcttccccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcac
atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc
gctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggaggtggccccggacttct
tcgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagc
agatggtggacgccagcaggcctgagctgctctaccgcaccgactttttccctggcctgggctggctgctgttggccgagctctgggc
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcaggggcgggcctgcatac
gccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctg
aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacgg
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt
ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggagggctatgatcctagctggaattag

Figure 10

GnT1 Arg120Ala, Cys121H

avipilviacdrstvr**ah**ldkllhyrpsaelfpiivsqdcgheetaqaiasygsavthirqpdlssiavppdhrkfqgyykiarhyrw
algqvfrqfrfpaavvveddlevapdffeyfratypllkadpslwcvsawndngkeqmvdasrpellyrtdffpglgwlllaelwa
elepkwpkafwddwmrrpeqrqgracirpeisrtmtfgrkgvshgqffdqhlkfiklnqqfvhftqldlsylqreaydrdflarvy
gapqlqvekvrtndrkelgevrvqytgrdsfkafakalgvmddlksgvpragyrgivtfqfpgrrvhlappptwegydpswn*

Gcggtgattcccatcctggtcatcgcctgtgaccgcagcactgttcgggcccacctagacaagctgctgcattatcggccctcggctg
agctcttccccatcatcgttagccaggactgcgggcacgaggagacggcccaggccatcgcctcctacggcagcgcggtcacgcac
atccggcagcccgacctgagcagcattgcggtgccgccggaccaccgcaagttccagggctactacaagatcgcgcgccactacc
gctgggcgctgggccaggtcttccggcagtttcgcttccccgcggccgtggtggtggaggatgacctggaggtggccccggacttct
tcgagtactttcgggccacctatccgctgctgaaggccgacccctccctgtggtgcgtctcggcctggaatgacaacggcaaggagc
agatggtggacgccagcaggcctgagctgctctaccgcaccgactttttccctggcctgggctggctgctgttggccgagctctgggc
tgagctggagcccaagtggccaaaggccttctgggacgactggatgcggcggccggagcagcggcaggggcgggcctgcatac
gccctgagatctcaagaacgatgacctttggccgcaagggtgtgagccacgggcagttctttgaccagcacctcaagtttatcaagctg
aaccagcagtttgtgcacttcacccagctggacctgtcttacctgcagcgggaggcctatgaccgagatttcctcgcccgcgtctacgg
tgctccccagctgcaggtggagaaagtgaggaccaatgaccggaaggagctgggggaggtgcgggtgcagtatacgggcaggga
cagcttcaaggctttcgccaaggctctgggtgtcatggatgaccttaagtcgggggttccgagagctggctaccggggtattgtcacctt
ccagttcccgggccgccgtgtccacctggcgcccccaccgacgtgggagggctatgatcctagctggaattag

Figure 11

Rat Liver ST3Gal III amino acid sequence:

*MGLLVFVRNLLLALCLFLVLGFLYYSAW*KLHLLQWEDSNSLILSLDSAGQTLGTEYDRL
GFLLKLDSKLPAELATKYANFSEGACKPGYASAMMTAIFPRFSKPAPMFLDDSFRKW
ARIREFVPPFGIKGQDNLIKAILSVTKEYRLTPALDSLHCRRCIIVGNGGVLANKSLGS
RIDDYDIVIRLNSAPVKGFEKDVGSKTTLRITYPEGAMQRPEQYERDSLFVLAGFKW
QDFKWLKYIVYKERVSASDGFWKSVATRVPKEPPEIRILNPYFIQEAAFTLIGLPFNN
GLMGRGNIPTLGSVAVTMALDGCDEVAVAGFGYDMNTPNAPLHYYETVRMAAIKE
SWTHNIQREKEFLRKLVKARVITDLSSGI

Figure 12

Full length UDP-N-acetylgalactosaminyltransferase 2 (GalNAcT2) nucleic acid and amino acid sequences

Amino acid sequence

```
Met Arg Arg Arg Ser Arg Met Leu Leu Cys Phe Ala Phe Leu Trp Val
1               5                   10                  15

Leu Gly Ile Ala Tyr Tyr Met Tyr Ser Gly Gly Gly Ser Ala Leu Ala
            20                  25                  30

Gly Gly Ala Gly Gly Gly Ala Gly Arg Lys Glu Asp Trp Asn Glu Ile
        35                  40                  45

Asp Pro Ile Lys Lys Lys Asp Leu His His Ser Asn Gly Glu Glu Lys
    50                  55                  60

Ala Gln Ser Met Glu Thr Leu Pro Pro Gly Lys Val Arg Trp Pro Asp
65                  70                  75                  80

Phe Asn Gln Glu Ala Tyr Val Gly Gly Thr Met Val Arg Ser Gly Gln
                85                  90                  95

Asp Pro Tyr Ala Arg Asn Lys Phe Asn Gln Val Glu Ser Asp Lys Leu
            100                 105                 110

Arg Met Asp Arg Ala Ile Pro Asp Thr Arg His Asp Gln Cys Gln Arg
        115                 120                 125

Lys Gln Trp Arg Val Asp Leu Pro Ala Thr Ser Val Val Ile Thr Phe
    130                 135                 140

His Asn Glu Ala Arg Ser Ala Leu Leu Arg Thr Val Val Ser Val Leu
145                 150                 155                 160

Lys Lys Ser Pro Pro His Leu Ile Lys Glu Ile Ile Leu Val Asp Asp
                165                 170                 175

Tyr Ser Asn Asp Pro Glu Asp Gly Ala Leu Leu Gly Lys Ile Glu Lys
            180                 185                 190

Val Arg Val Leu Arg Asn Asp Arg Arg Glu Gly Leu Met Arg Ser Arg
        195                 200                 205

Val Arg Gly Ala Asp Ala Ala Gln Ala Lys Val Leu Thr Phe Leu Asp
    210                 215                 220

Ser His Cys Glu Cys Asn Glu His Trp Leu Glu Pro Leu Leu Glu Arg
225                 230                 235                 240

Val Ala Glu Asp Arg Thr Arg Val Val Ser Pro Ile Ile Asp Val Ile
                245                 250                 255

Asn Met Asp Asn Phe Gln Tyr Val Gly Ala Ser Ala Asp Leu Lys Gly
            260                 265                 270

Gly Phe Asp Trp Asn Leu Val Phe Lys Trp Asp Tyr Met Thr Pro Glu
        275                 280                 285
```

FIG. 13A (1/2)

Gln Arg Arg Ser Arg Gln Gly Asn Pro Val Ala Pro Ile Lys Thr Pro
290 295 300

Met Ile Ala Gly Gly Leu Phe Val Met Asp Lys Phe Tyr Phe Glu Glu
305 310 315 320

Leu Gly Lys Tyr Asp Met Met Met Asp Val Trp Gly Gly Glu Asn Leu
325 330 335

Glu Ile Ser Phe Arg Val Trp Gln Cys Gly Gly Ser Leu Glu Ile Ile
340 345 350

Pro Cys Ser Arg Val Gly His Val Phe Arg Lys Gln His Pro Tyr Thr
355 360 365

Phe Pro Gly Gly Ser Gly Thr Val Phe Ala Arg Asn Thr Arg Arg Ala
370 375 380

Ala Glu Val Trp Met Asp Glu Tyr Lys Asn Phe Tyr Tyr Ala Ala Val
385 390 395 400

Pro Ser Ala Arg Asn Val Pro Tyr Gly Asn Ile Gln Ser Arg Leu Glu
405 410 415

Leu Arg Lys Lys Leu Ser Cys Lys Pro Phe Lys Trp Tyr Leu Glu Asn
420 425 430

Val Tyr Pro Glu Leu Arg Val Pro Asp His Gln Asp Ile Ala Phe Gly
435 440 445

Ala Leu Gln Gln Gly Thr Asn Cys Leu Asp Thr Leu Gly His Phe Ala
450 455 460

Asp Gly Val Val Gly Val Tyr Glu Cys His Asn Ala Gly Gly Asn Gln
465 470 475 480

Glu Trp Ala Leu Thr Lys Glu Lys Ser Val Lys His Met Asp Leu Cys
485 490 495

Leu Thr Val Val Asp Arg Ala Pro Gly Ser Leu Ile Lys Leu Gln Gly
500 505 510

Cys Arg Glu Asn Asp Ser Arg Gln Lys Trp Glu Gln Ile Glu Gly Asn
515 520 525

Ser Lys Leu Arg His Val Gly Ser Asn Leu Cys Leu Asp Ser Arg Thr
530 535 540

Ala Lys Ser Gly Gly Leu Ser Val Glu Val Cys Gly Pro Ala Leu Ser
545 550 555 560

Gln Gln Trp Lys Phe Thr Leu Asn Leu Gln Gln
565 570

FIG. 13A (2/2)

Nucleic acid sequence

```
atgcggcggc gctcgcggat gctgctctgc ttcgccttcc tgtgggtgct gggcatcgcc      60
tactacatgt actcgggggg cggctctgcg ctggccgggg gcgcgggcgg cggcgccggc     120
aggaaggagg actggaatga aattgacccc attaaaaaga aagaccttca tcacagcaat     180
ggagaagaga aagcacaaag catggagacc ctccctccag ggaaagtacg gtggccagac     240
tttaaccagg aagcttatgt tggagggacg atggtccgct ccgggcagga cccttacgcc     300
cgcaacaagt tcaaccaggt ggagagtgat aagcttcgaa tggacagagc catccctgac     360
acccggcatg accagtgtca gcggaagcag tggcgggtgg atctgccggc caccagcgtg     420
gtgatcacgt ttcacaatga agccaggtcg gccctactca ggaccgtggt cagcgtgctt     480
aagaaaagcc cgccccatct cataaaagaa atcatcttgg tggatgacta cagcaatgat     540
cctgaggacg gggctctctt ggggaaaatt gagaaagtgc gagttcttag aaatgatcga     600
cgagaaggcc tcatgcgctc acgggttcgg ggggccgatg ctgcccaagc caaggtcctg     660
accttcctgg acagtcactg cgagtgtaat gagcactggc tggagcccct cctggaaagg     720
gtggcggagg acaggactcg ggttgtgtca cccatcatcg atgtcattaa tatggacaac     780
tttcagtatg tgggggcatc tgctgacttg aagggcggtt ttgattggaa cttggtattc     840
aagtgggatt acatgacgcc tgagcagaga aggtcccggc aggggaaccc agtcgcccct     900
ataaaaaccc ccatgattgc tggtgggctg tttgtgatgg ataagttcta ttttgaagaa     960
ctggggaagt acgacatgat gatggatgtg tggggaggag agaacctaga gatctcgttc    1020
cgcgtgtggc agtgtggtgg cagcctggag atcatcccgt gcagccgtgt gggacacgtg    1080
ttccggaagc agcaccccta cacgttcccg ggtggcagtg gcactgtctt tgcccgaaac    1140
acccgccggg cagcagaggt ctggatggat gaatacaaaa atttctatta tgcagcagtg    1200
ccttctgcta gaaacgttcc ttatggaaat attcagagca gattggagct taggaagaaa    1260
ctcagctgca agcctttcaa atggtacctt gaaaatgtct atccagagtt aagggttcca    1320
gaccatcagg atatagcttt tggggccttg cagcagggaa ctaactgcct cgacactttg    1380
ggacactttg ctgatggtgt ggttggagtt tatgaatgtc acaatgctgg gggaaaccag    1440
gaatgggcct tgacgaagga gaagtcggtg aagcacatgg atttgtgcct tactgtggtg    1500
gaccgggcac cgggctctct tataaagctg cagggctgcc gagaaaatga cagcagacag    1560
aaatgggaac agatcgaggg caactccaag ctgaggcacg tgggcagcaa cctgtgcctg    1620
gacagtcgca cggccaagag cgggggccta agcgtggagg tgtgtggccc ggccctttcg    1680
cagcagtgga agttcacgct caacctgcag cag                                 1713
```

Figure 13B

Δ51 UDP-N-acetylgalactosaminyltransferase 2, GalNAcT2, nucleic acid and amino acid sequences

Amino acid sequence

```
Lys Lys Lys Asp Leu His His Ser Asn Gly Glu Glu Lys Ala Gln Ser
1               5                   10                  15

Met Glu Thr Leu Pro Pro Gly Lys Val Arg Trp Pro Asp Phe Asn Gln
            20                  25                  30

Glu Ala Tyr Val Gly Gly Thr Met Val Arg Ser Gly Gln Asp Pro Tyr
        35                  40                  45

Ala Arg Asn Lys Phe Asn Gln Val Glu Ser Asp Lys Leu Arg Met Asp
    50                  55                  60

Arg Ala Ile Pro Asp Thr Arg His Asp Gln Cys Gln Arg Lys Gln Trp
65                  70                  75                  80

Arg Val Asp Leu Pro Ala Thr Ser Val Val Ile Thr Phe His Asn Glu
                85                  90                  95

Ala Arg Ser Ala Leu Leu Arg Thr Val Val Ser Val Leu Lys Lys Ser
            100                 105                 110

Pro Pro His Leu Ile Lys Glu Ile Ile Leu Val Asp Asp Tyr Ser Asn
        115                 120                 125

Asp Pro Glu Asp Gly Ala Leu Leu Gly Lys Ile Glu Lys Val Arg Val
    130                 135                 140

Leu Arg Asn Asp Arg Arg Glu Gly Leu Met Arg Ser Arg Val Arg Gly
145                 150                 155                 160

Ala Asp Ala Ala Gln Ala Lys Val Leu Thr Phe Leu Asp Ser His Cys
                165                 170                 175

Glu Cys Asn Glu His Trp Leu Glu Pro Leu Leu Glu Arg Val Ala Glu
            180                 185                 190

Asp Arg Thr Arg Val Val Ser Pro Ile Ile Asp Val Ile Asn Met Asp
        195                 200                 205

Asn Phe Gln Tyr Val Gly Ala Ser Ala Asp Leu Lys Gly Gly Phe Asp
    210                 215                 220

Trp Asn Leu Val Phe Lys Trp Asp Tyr Met Thr Pro Glu Gln Arg Arg
225                 230                 235                 240

Ser Arg Gln Gly Asn Pro Val Ala Pro Ile Lys Thr Pro Met Ile Ala
                245                 250                 255

Gly Gly Leu Phe Val Met Asp Lys Phe Tyr Phe Glu Glu Leu Gly Lys
            260                 265                 270

Tyr Asp Met Met Met Asp Val Trp Gly Gly Glu Asn Leu Glu Ile Ser
        275                 280                 285

Phe Arg Val Trp Gln Cys Gly Gly Ser Leu Glu Ile Ile Pro Cys Ser
```

FIG. 14A (1/2)

290 295 300

Arg Val Gly His Val Phe Arg Lys Gln His Pro Tyr Thr Phe Pro Gly
305 310 315 320

Gly Ser Gly Thr Val Phe Ala Arg Asn Thr Arg Arg Ala Ala Glu Val
325 330 335

Trp Met Asp Glu Tyr Lys Asn Phe Tyr Tyr Ala Ala Val Pro Ser Ala
340 345 350

Arg Asn Val Pro Tyr Gly Asn Ile Gln Ser Arg Leu Glu Leu Arg Lys
355 360 365

Lys Leu Ser Cys Lys Pro Phe Lys Trp Tyr Leu Glu Asn Val Tyr Pro
370 375 380

Glu Leu Arg Val Pro Asp His Gln Asp Ile Ala Phe Gly Ala Leu Gln
385 390 395 400

Gln Gly Thr Asn Cys Leu Asp Thr Leu Gly His Phe Ala Asp Gly Val
405 410 415

Val Gly Val Tyr Glu Cys His Asn Ala Gly Gly Asn Gln Glu Trp Ala
420 425 430

Leu Thr Lys Glu Lys Ser Val Lys His Met Asp Leu Cys Leu Thr Val
435 440 445

Val Asp Arg Ala Pro Gly Ser Leu Ile Lys Leu Gln Gly Cys Arg Glu
450 455 460

Asn Asp Ser Arg Gln Lys Trp Glu Gln Ile Glu Gly Asn Ser Lys Leu
465 470 475 480

Arg His Val Gly Ser Asn Leu Cys Leu Asp Ser Arg Thr Ala Lys Ser
485 490 495

Gly Gly Leu Ser Val Glu Val Cys Gly Pro Ala Leu Ser Gln Gln Trp
500 505 510

Lys Phe Thr Leu Asn Leu Gln Gln
515 520

FIG. 14A (2/2)

Nucleic acid sequence

aaaaagaaag accttcatca cagcaatgga gaagagaaag cacaaagcat ggagaccctc 60
cctccaggga aagtacggtg gccagacttt aaccaggaag cttatgttgg agggacgatg 120
gtccgctccg ggcaggaccc ttacgcccgc aacaagttca accaggtgga gagtgataag 180
cttcgaatgg acagagccat ccctgacacc cggcatgacc agtgtcagcg gaagcagtgg 240
cgggtggatc tgccggccac cagcgtggtg atcacgtttc acaatgaagc caggtcggcc 300
ctactcagga ccgtggtcag cgtgcttaag aaaagcccgc cccatctcat aaaagaaatc 360
atcttggtgg atgactacag caatgatcct gaggacgggg ctctcttggg gaaaattgag 420
aaagtgcgag ttcttagaaa tgatcgacga gaaggcctca tgcgctcacg ggttcggggg 480
gccgatgctg cccaagccaa ggtcctgacc ttcctggaca gtcactgcga gtgtaatgag 540
cactggctgg agcccctcct ggaaagggtg gcggaggaca ggactcgggt tgtgtcaccc 600
atcatcgatg tcattaatat ggacaacttt cagtatgtgg gggcatctgc tgacttgaag 660
ggcggttttg attggaactt ggtattcaag tgggattaca tgacgcctga gcagagaagg 720
tcccggcagg ggaacccagt cgcccctata aaaacccccca tgattgctgg tgggctgttt 780
gtgatggata agttctattt tgaagaactg gggaagtacg acatgatgat ggatgtgtgg 840
ggaggagaga acctagagat ctcgttccgc gtgtggcagt gtggtggcag cctggagatc 900
atcccgtgca gccgtgtggg acacgtgttc cggaagcagc acccctacac gttcccgggt 960
ggcagtggca ctgtctttgc ccgaaacacc cgccgggcag cagaggtctg gatggatgaa 1020
tacaaaaatt tctattatgc agcagtgcct tctgctagaa acgttcctta tggaaatatt 1080
cagagcagat tggagcttag gaagaaactc agctgcaagc ctttcaaatg gtaccttgaa 1140
aatgtctatc cagagttaag ggttccagac catcaggata tagcttttgg ggccttgcag 1200
cagggaacta actgcctcga cactttggga cactttgctg atggtgtggt tggagtttat 1260
gaatgtcaca atgctggggg aaaccaggaa tgggccttga cgaaggagaa gtcggtgaag 1320
cacatggatt tgtgccttac tgtggtggac cgggcaccgg gctctcttat aaagctgcag 1380
ggctgccgag aaaatgacag cagacagaaa tgggaacaga tcgagggcaa ctccaagctg 1440
aggcacgtgg gcagcaacct gtgcctggac agtcgcacgg ccaagagcgg gggcctaagc 1500
gtggaggtgt gtggcccggc cctttcgcag cagtggaagt tcacgctcaa cctgcagcag 1560

Figure 14B

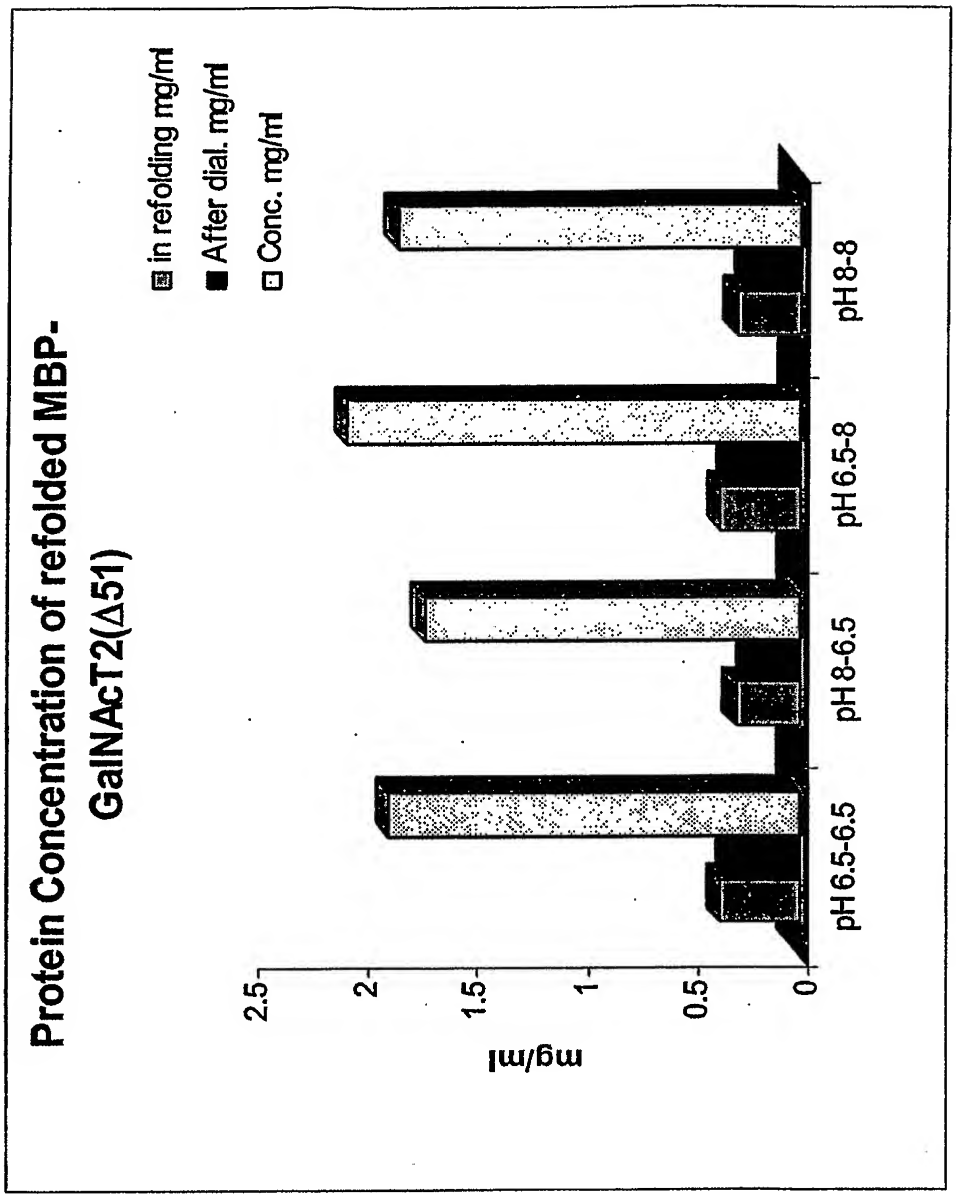

Protein Concentration of refolded MBP-GalNAcT2(Δ51)
mg/ml
2.5
2
1.5
1
0.5
0
pH 6.5-6.5
pH 8-6.5
pH 6.5-8
pH 8-8
in refolding mg/ml
After dial. mg/ml
Conc. mg/ml

Figure 15

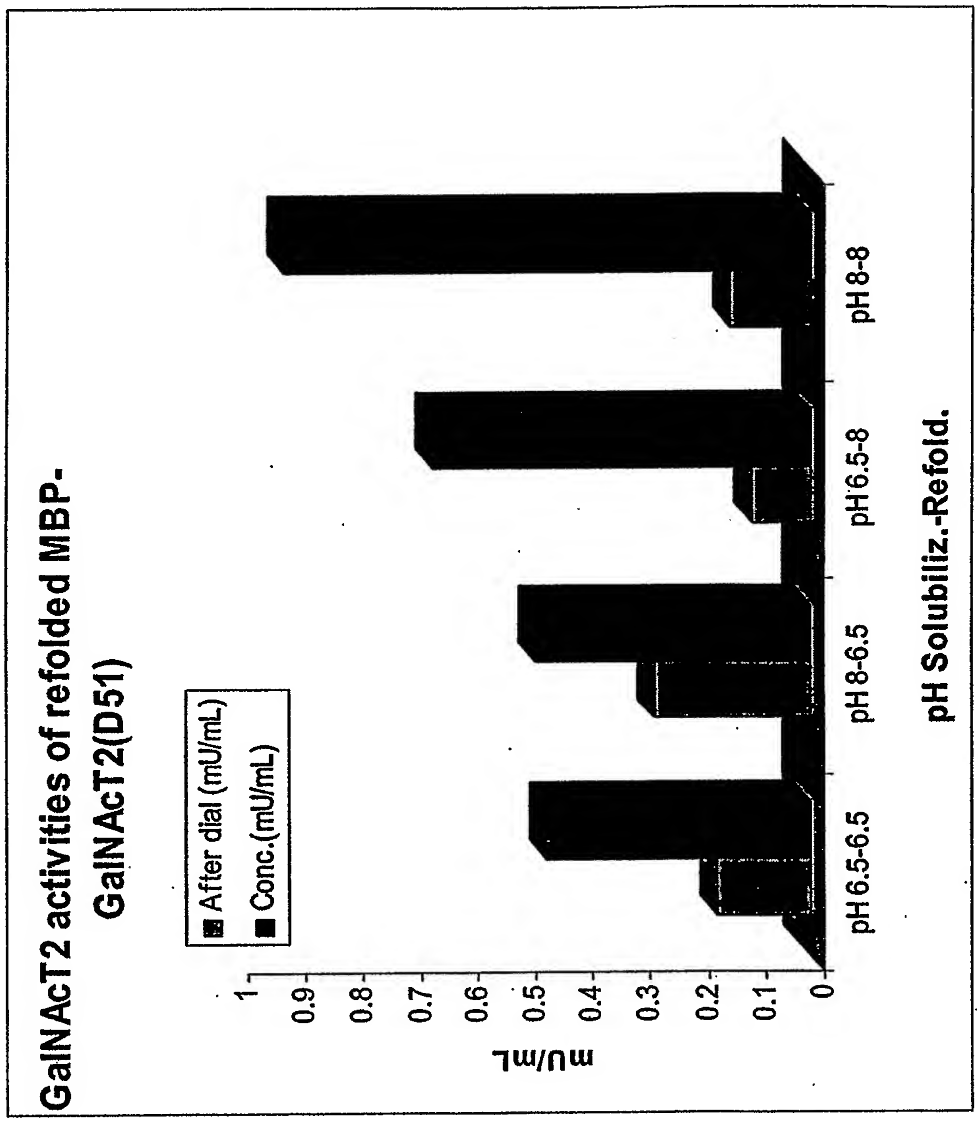

GalNAcT2 activities of refolded MBP-GalNAcT2(D51)
After dial (mU/mL)
Conc.(mU/mL)
mU/mL
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pH 6.5-6.5
pH 8-6.5
pH 6.5-8
pH 8-8
pH Solubiliz.-Refold.

Figure 16

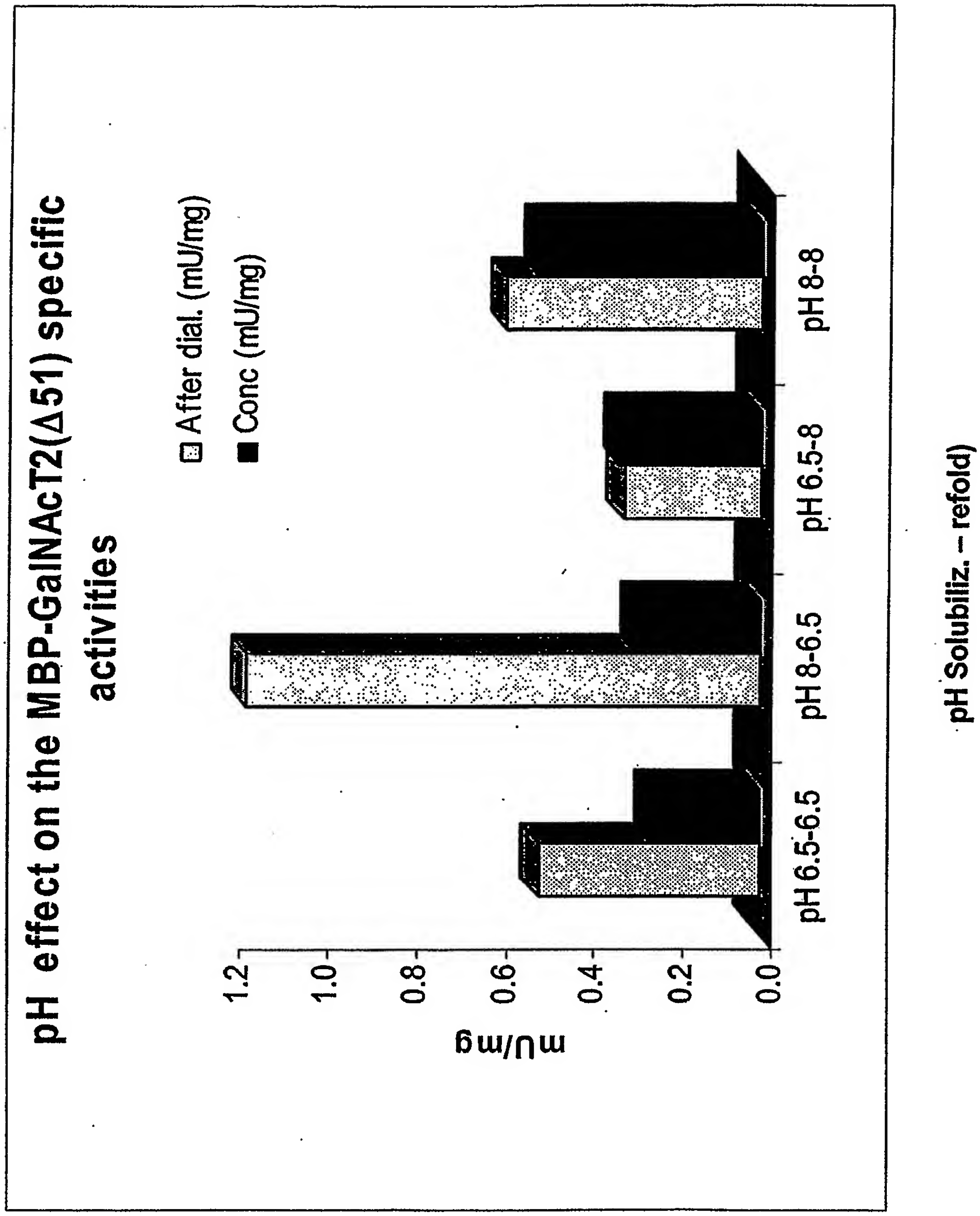

pH effect on the MBP-GalNAcT2(Δ51) specific activities
After dial. (mU/mg)
Conc (mU/mg)
mU/mg
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pH 6.5-6.5
pH 8-6.5
pH 6.5-8
pH 8-8
pH Solubiliz. -- refold)

Figure 17

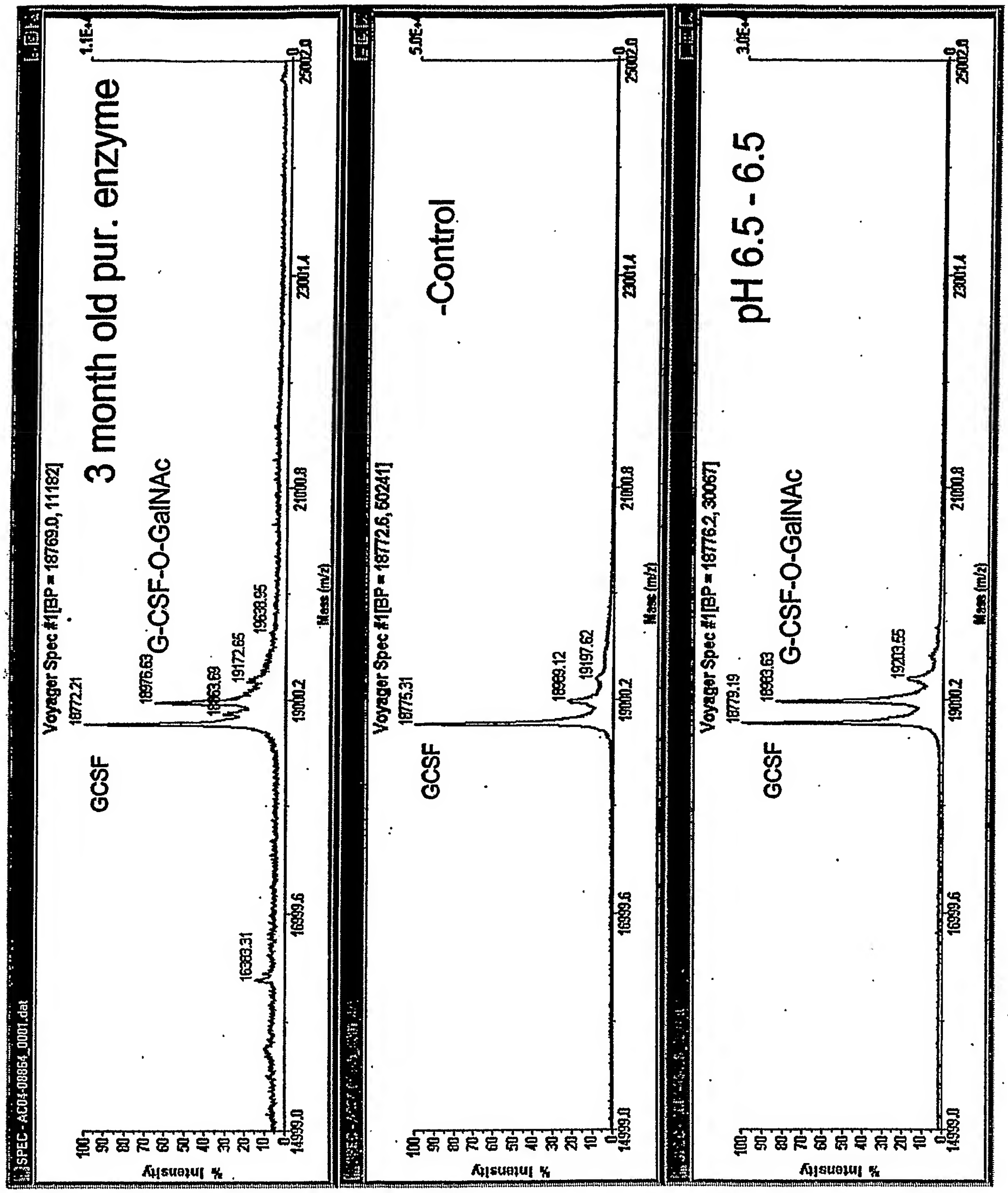

3 month old pur. enzyme
GCSF
G-CSF-O-GalNAc
Voyager Spec #1[BP = 18769.0, 11182]
-Control
Voyager Spec #1[BP = 18772.6, 50241]
pH 6.5 - 6.5
Voyager Spec #1[BP = 18776.2, 30067]
% Intensity
Mass (m/z)

Figure 18A

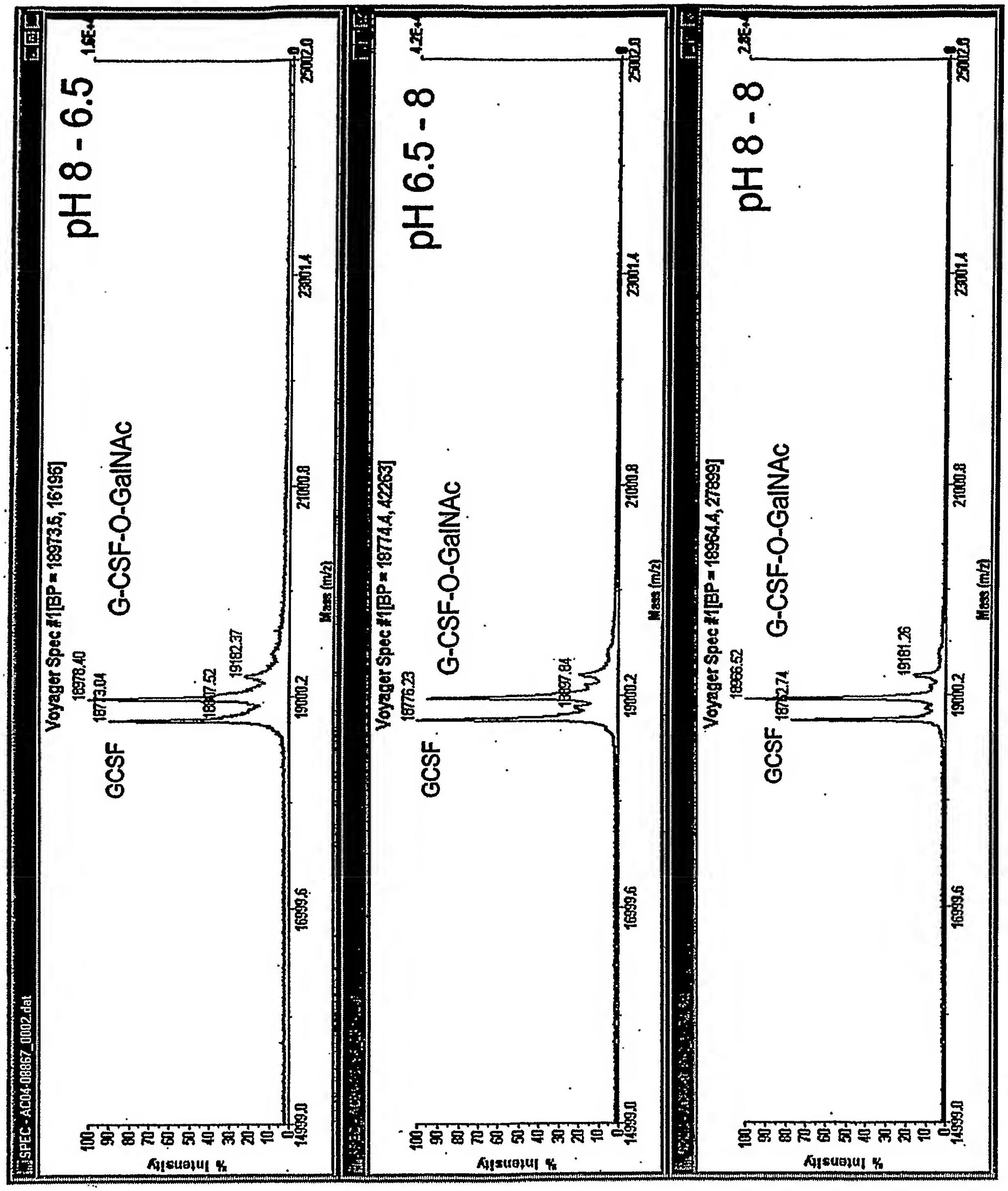

pH 8 - 6.5
G-CSF-O-GalNAc
GCSF
Voyager Spec #1[BP = 18973.5, 16196]
pH 6.5 - 8
G-CSF-O-GalNAc
GCSF
Voyager Spec #1[BP = 18774.4, 42263]
pH 8 - 8
G-CSF-O-GalNAc
GCSF
Voyager Spec #1[BP = 18964.4, 27899]
% Intensity
Mass (m/z)
14999.0
16999.6
19000.2
21000.8
23001.4
25002.0

Figure 18B

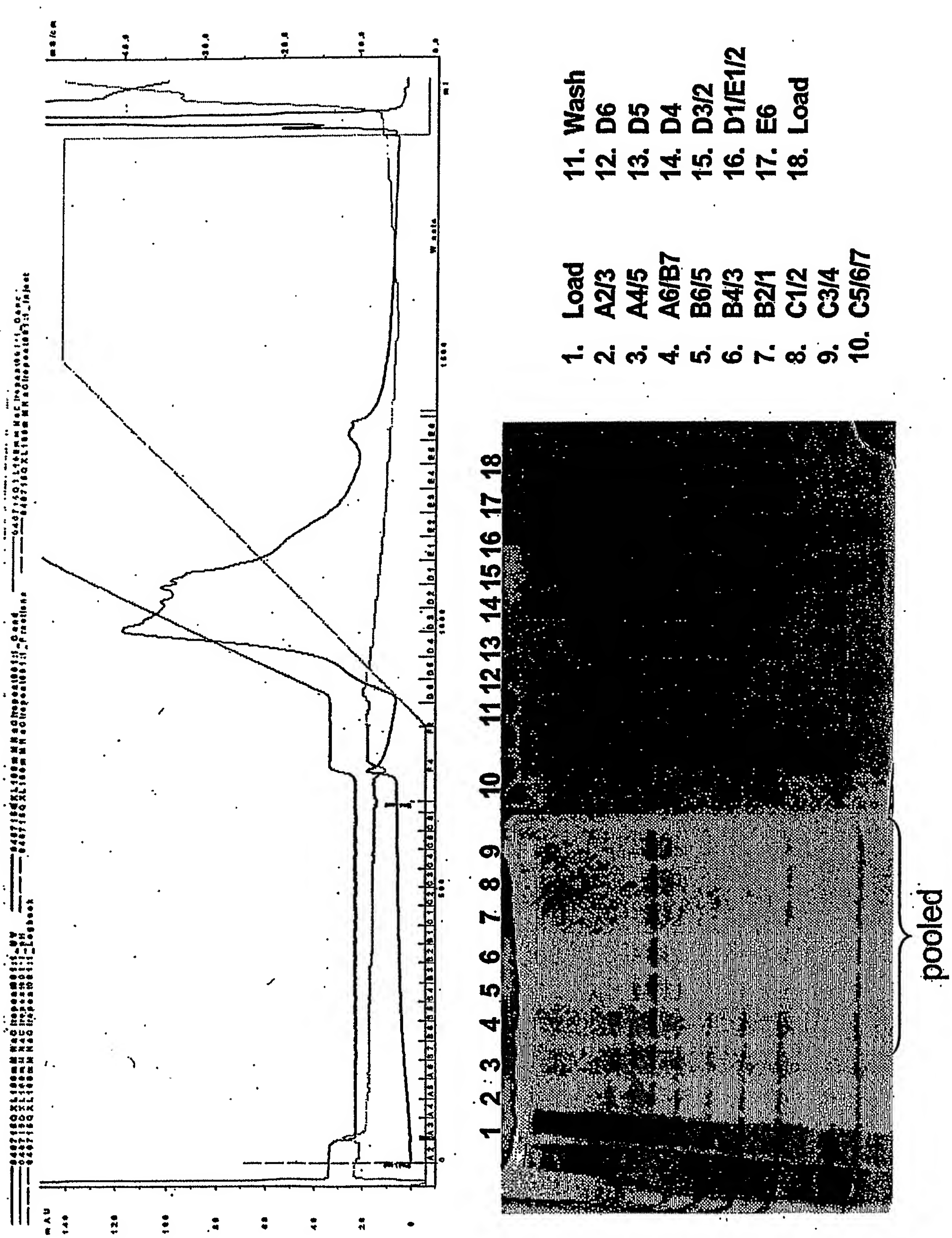

1. Load
2. A2/3
3. A4/5
4. A6/B7
5. B6/5
6. B4/3
7. B2/1
8. C1/2
9. C3/4
10. C5/6/7
11. Wash
12. D6
13. D5
14. D4
15. D3/2
16. D1/E1/2
17. E6
18. Load
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pooled

FIG. 19

	Volume (mL)	Activity (U/L)	A280
Load	890	1.5	0.110
$FT_{A4\text{-}C4}$	670	**9.2**	NA
$FT_{C5\text{-}C7}$	120	1.0	0
Wash	138	3.6	0.100
D6	45	4.5	0
D5	45	2.4	0.026
D4	45	2.0	0.108
D3/2	90	1.1	0.179
E6		0.0	0.017

FIG. 20

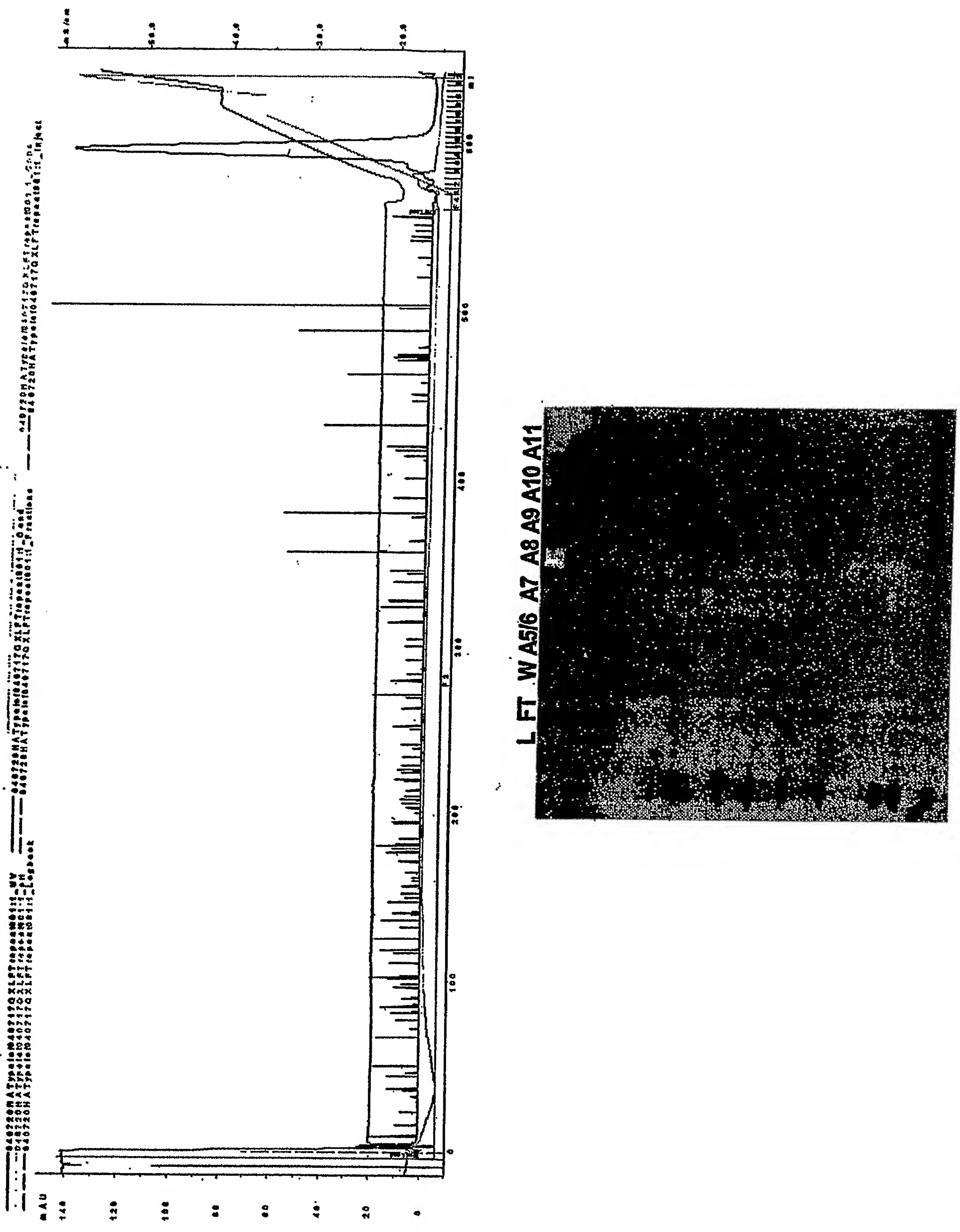


FIG. 21

Pooled A4-C4 and adjusted pH to 7.0 using 1mM HCl 670mL load pH 7.0 16mS/cm

	Volume (mL)	Activity (U/L) pre-dialysis	Activity (U/L) post-dialysis	A280	A280/ 1.51 (mg/mL)	Activity (U)	Mass (mg)	Specific Activity (U/mg)
Load	670	9.2	NA	NA				
FT	670	0.0	NA	0.122				
Wash	9	2.9	NA	-0.013				
A5/6	6	1.1	NA	-0.005				
A7	3	0.1						
A8	3	1.3						
A9	3	4.6	19.3 (13mL)	0.180	0.119	0.25	1.55	0.16
A10	3	2.4						
A11	3	0.4						

FIG. 22

COMPARISON of MBP with MBP-SBD$_{39}$ tag in ST3Gal3 in Cyclodextrin column purification

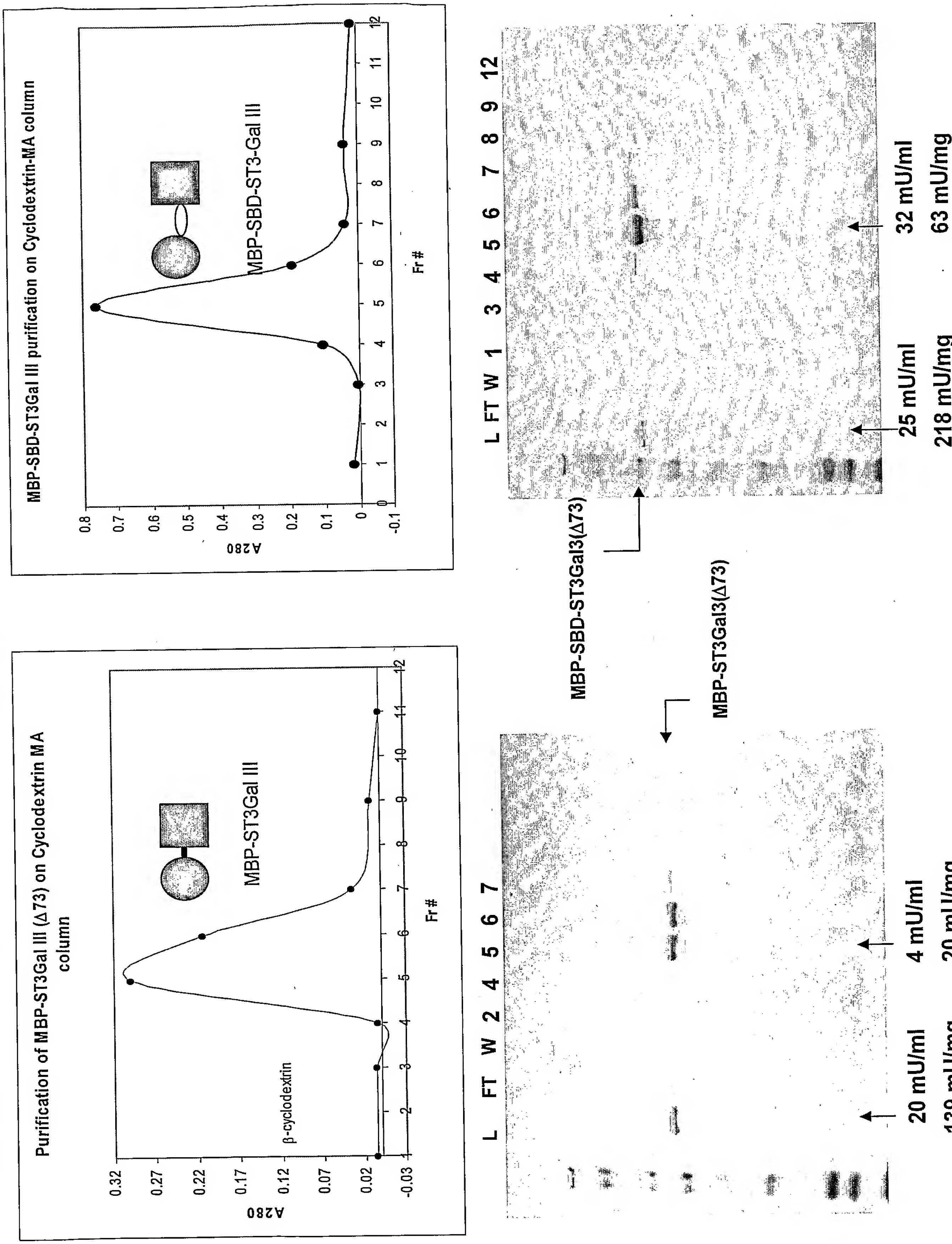


Figure 24

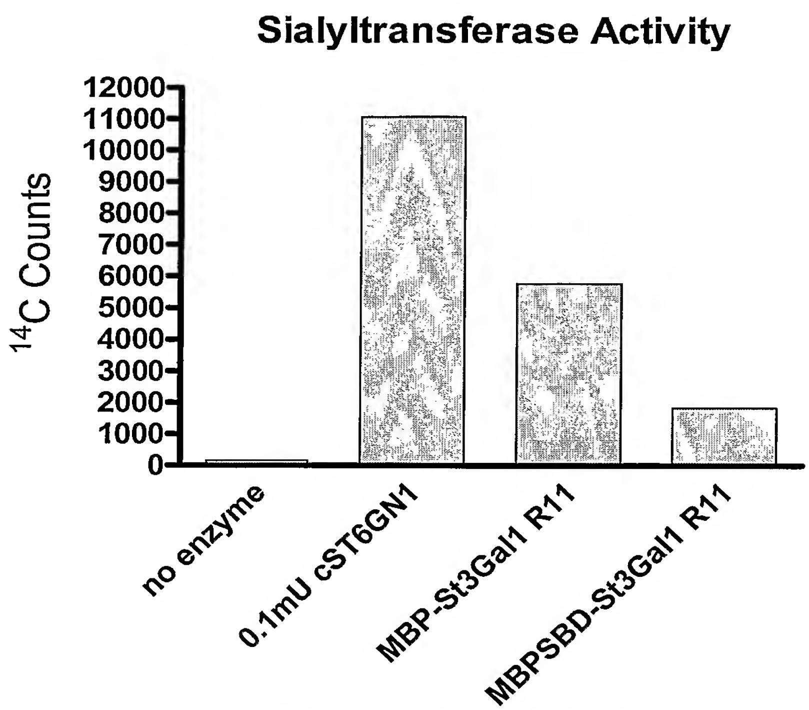
a) MBP-pST3Gal1 fusion protein

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF
WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGSELSENFKKLMKYPYR
PCTCTRCIEEQRVSAWFDERFNRSMQPLLTAKNAHLEEDTYKWWLRLQREKQPNNLNDTIRELFQVVP
GNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPQIDSHDFVLRMNKAPTEGFEADVGSKTTHHFVYPE
SFRELAQEVSMILVPFKTTDLEWVISATTGRISHTYVPVPAKIKVKKEKILIYHPAFIKYVFDRWLQGH
GRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDFESNVTTILASIN
KIRIFKGR

b) MBPSBD-pST3Gal1 fusion protein

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF
WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGSIVATGGTTTTATPTG
SGSVTSTSKTTATASKTSTSTSSTSCTTPTAVAVTFDLTATTTYGENIYLVGSISQLGDWETSDGIALSAD
KYTSSDPLWYVTVTLPAGESFEYKFIRIESDDSVEWESDPNREYTVPQACGTSTATVTDTWRGSELSEN
FKKLMKYPYRPCTCTRCIEEQRVSAWFDERFNRSMQPLLTAKNAHLEEDTYKWWLRLQREKQPNNLN
DTIRELFQVVPGNVDPLLEKRLVSCRRCAVVGNSGNLKESYYGPQIDSHDFVLRMNKAPTEGFEADVG
SKTTHHFVYPESFRELAQEVSMILVPFKTTDLEWVISATTGRISHTYVPVPAKIKVKKEKILIYHPAFIK
YVFDRWLQGHGRYPSTGILSVIFSLHICDEVDLYGFGADSKGNWHHYWENNPSAGAFRKTGVHDGDF
ESNVTTILASINKIRIFKGR

Figure 25



Test pST3Gal1 for activity after Hampton refold

Figure 26

A.

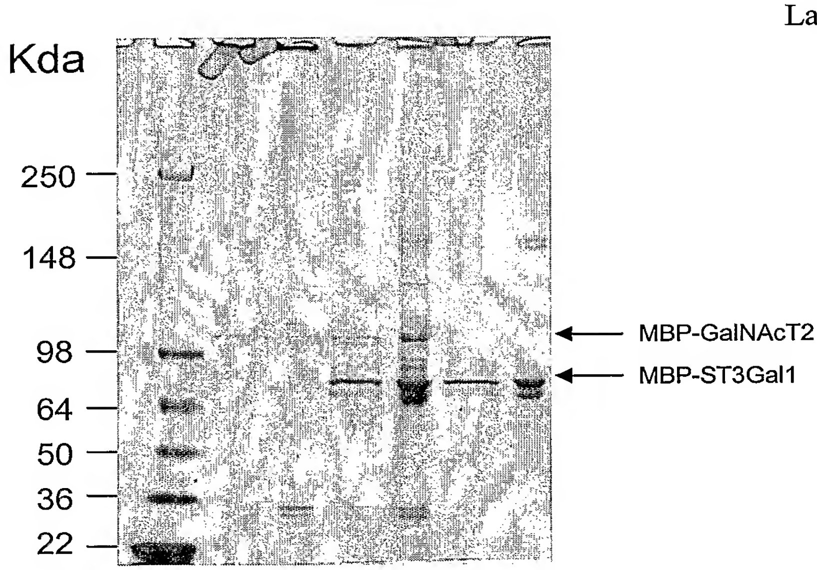
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF
WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGS**SEHLDKVPRTPGAL**
STRKTPMATGAVPAKKKVVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEEYSLDMSSLQT
NCSASVKIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSLVQKVVTRFP
PVRQQQLLLASLPTGYSKCITCAVVGNGGILNDSRVGREIDSHDYVFRLSGAVIKGYEQDVGTRT
SFYGFTAFSLTQSILILGRRGFQHVPLGKDVRYLHFLEGTRNYEWLEAMFLNQTLAKTHLSWFR
HRPQEAFRNALDLDRYLLLHPDFLRYMKNRFLRSKTLDTAXWRIYRPTTGALLLLTALHLCDKV
SAYGFITEGHERFSDHYYDTSWKRLIFYINHDFRLERMVWKRLHDEGIIWLYQRPQSDKAKN

B.

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRF
GGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALD
KELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH
MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAF
WYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFGS**KEPQTKPSRHQRTE**
NIKERSLQSLAKPKSQAPTRARRTTIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK
VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNGGQTRKLTA
SRTVSEKHQGKAATTAKTLIPKSQHRMLAPTGAVSTRTRQKGVTTAVIPPKEKKPQATPPPAPFQ
SPTTQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSVKIKASKSLWLQKLFLPNLTLFLDS
RHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVTRFPPVPQQQLLLASLPAGSLRCITCAVVGNG
GILNNSHMGQEIDSHDYVFRLSGALIKGYEQDVGTRTSFYGFTAFSLTQSLLILGNRGFKNVPLGK
DVRYLHFLEGTRDYEWLEALLMNQTVMSKNLFWFRHRPQEAFREALHMDRYLLLHPDFLRYM
KNRFLRSKTLDGAHWRIYRPTTGALLLLTALQLCDQVSAYGFITEGHERFSDHYYDTSWKRLIFY
INHDFKLEREVWKRLHDEGIIRLYQRPGPGTAKAKN

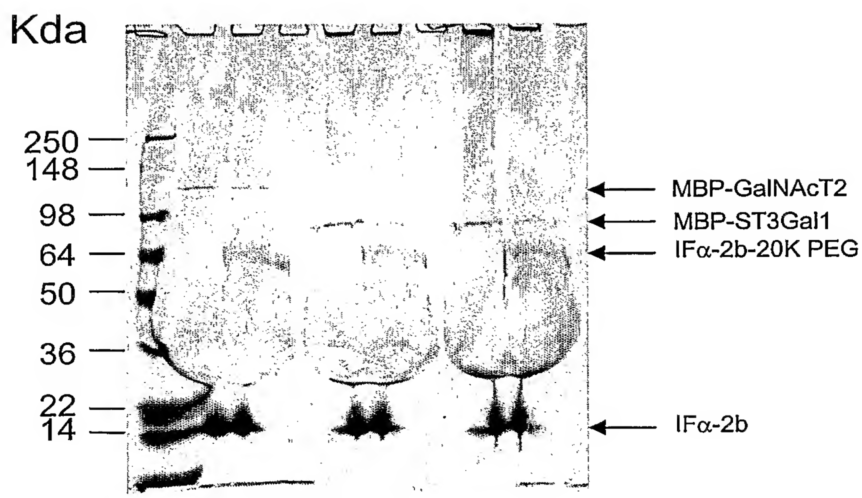
Figure 27

A.



Lanes: 1) See Blue Plus 2
2) MBP-GalNAcT2
3) MBP-GalNAcT2 (conc)
4) Co-refold
5) Co-refold (conc)
6) MBP-ST3Gal1
7) MBP-ST3Gal1 (conc)

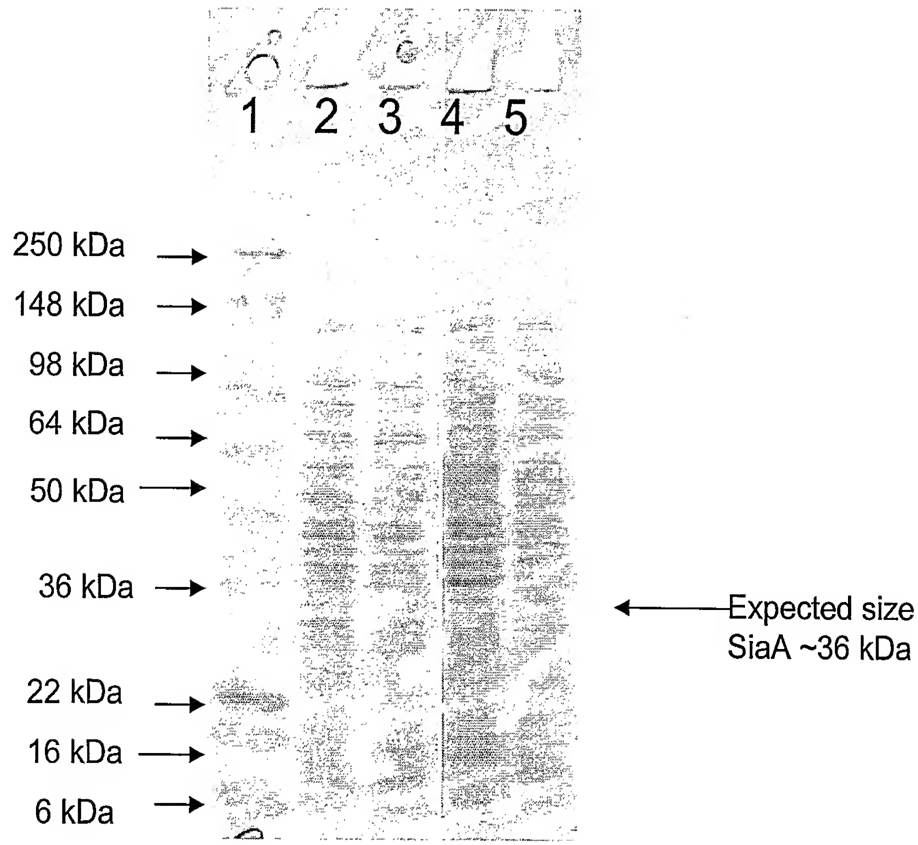
B.



Lanes:
1) See Blue Plus 2
2) Control enzymes 0hrs
3) Control enzymes 16hrs

4) Separate refold 0hrs
5) Separate refold 16hrs
6) Co-refold 0hrs
7) Co-refolded 16hrs

Figure 28: Induced SiaA expression in E.coli

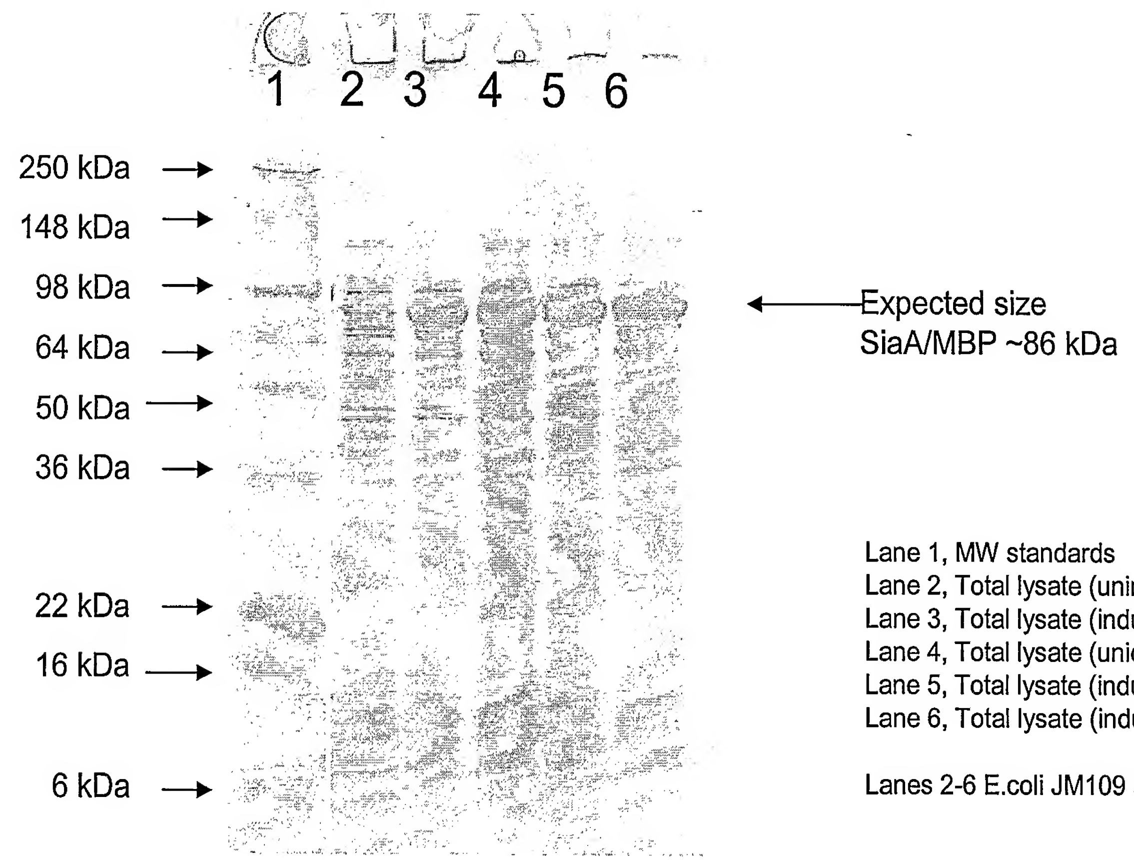


Lane 1, MW standards
Lane 2, Total lysate (uninduced)
Lane 3, Total lysate (induced)
Lane 4, Total lysate (uninduced)
Lane 5, Total lysate (induced)

Lanes 2-3 E.coli JM109 stain
Lanes 4-5 E.coli W3110 strain

There is no obvious inducible band at the expected Mass of 36 kDa for the native SiaA protein.

Figure 29: Induced SiaA/MBP expression in E.coli



Lane 1, MW standards
Lane 2, Total lysate (uninduced) colony A
Lane 3, Total lysate (induced) colony A
Lane 4, Total lysate (uniduced) colony B
Lane 5, Total lysate (induced) colony B
Lane 6, Total lysate (induced) colony C

Lanes 2-6 E.coli JM109 stain

High level production of SiaA/MBP even in absence of IPTG induction. Compare to figure X where SiaA production is not obvious. The presence of the fusion partner (MBP) drives high levels of expression.

Figure 30

```
  1 mkfrepllgg saampgaslq racrllvavc alhlgvtlvy ylagrdlrrl pqlvgvhppl
 61 qgsshgaaai gqpsgelrlr gvappppIqn sskprsraps nldayshpgp gpgpgsnlts
121 apvpstttrs ltacpeespl lvgpmliefn ipvdlklieq qnpkvklggr ytpmdcisph
181 kvaiiilfrn rqehlkywly ylhpmvqrqq ldygiyvinq agesmfnrak llnvgfkeal
241 kdydyncfvf sdvdlipmnd hntyrcfsqp rhisvamdkf gfslpyvqyf ggvsalskqq
301 flsingfpnn ywgwggeddd iynrlafrgm svsrpnavig kcrmirhsrd kknepnpqrf
361 driahtketm lsdglnslty mvlevqrypl ytkitvdigt ps
```

Bovine (b) GalT1 (β1,4GalT1) constructs

UDP-galactose β-N-acetylglucosaminide β4-galactosyltransferase (EC 2.4.1.38)

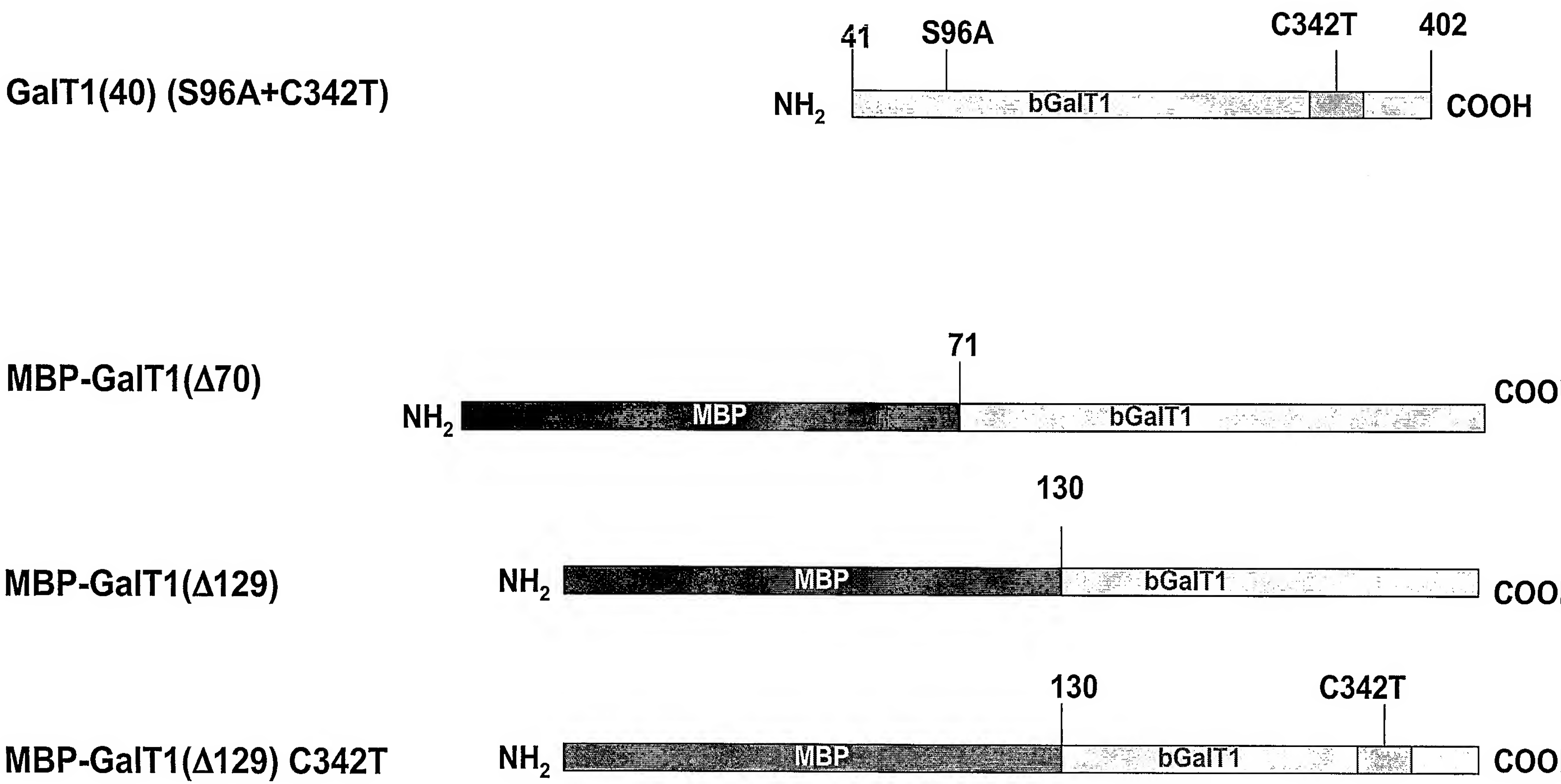
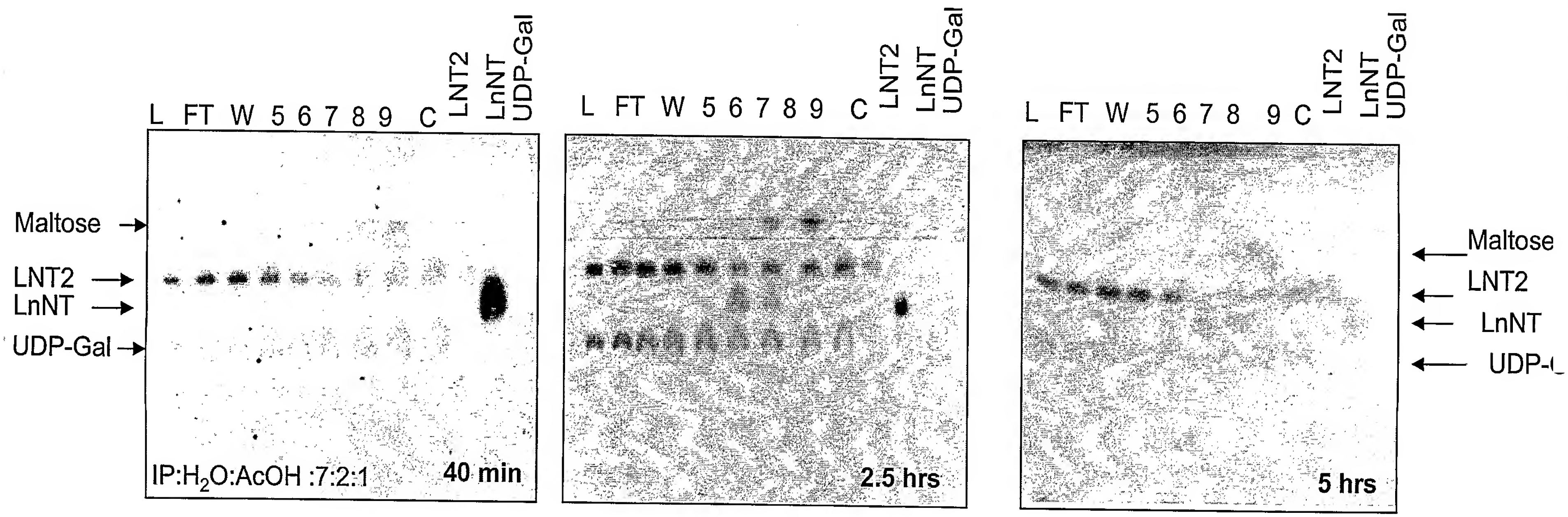


Figure 31

GalT1 TLC assay



LnNT reactions with

L: Loaded sample (refolded, dialyzed MBP-GalT1(70) onto amylose column)
FT: Amylose column Flow trough
W: Amylose column wash
5: Maltose Eluted Fr # 5
6: Maltose Eluted Fr # 6
7: Maltose Eluted Fr # 7
8: Maltose Eluted Fr # 8
9: Maltose Eluted Fr # 9
C: Control with water

Figure 32

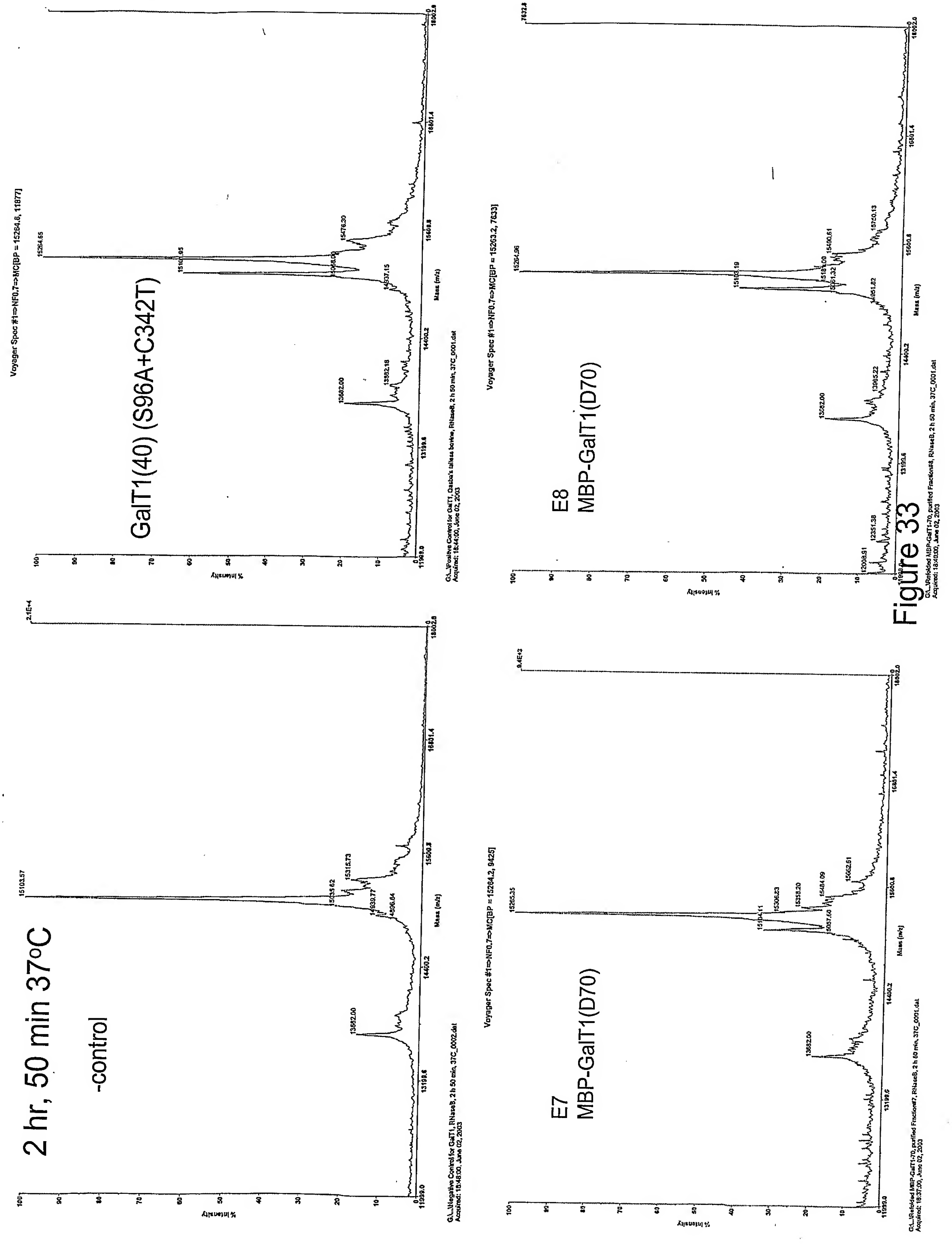

2 hr, 50 min 37°C
-control
GalT1(40) (S96A+C342T)
E7
MBP-GalT1(D70)
E8
MBP-GalT1(D70)

Figure 33

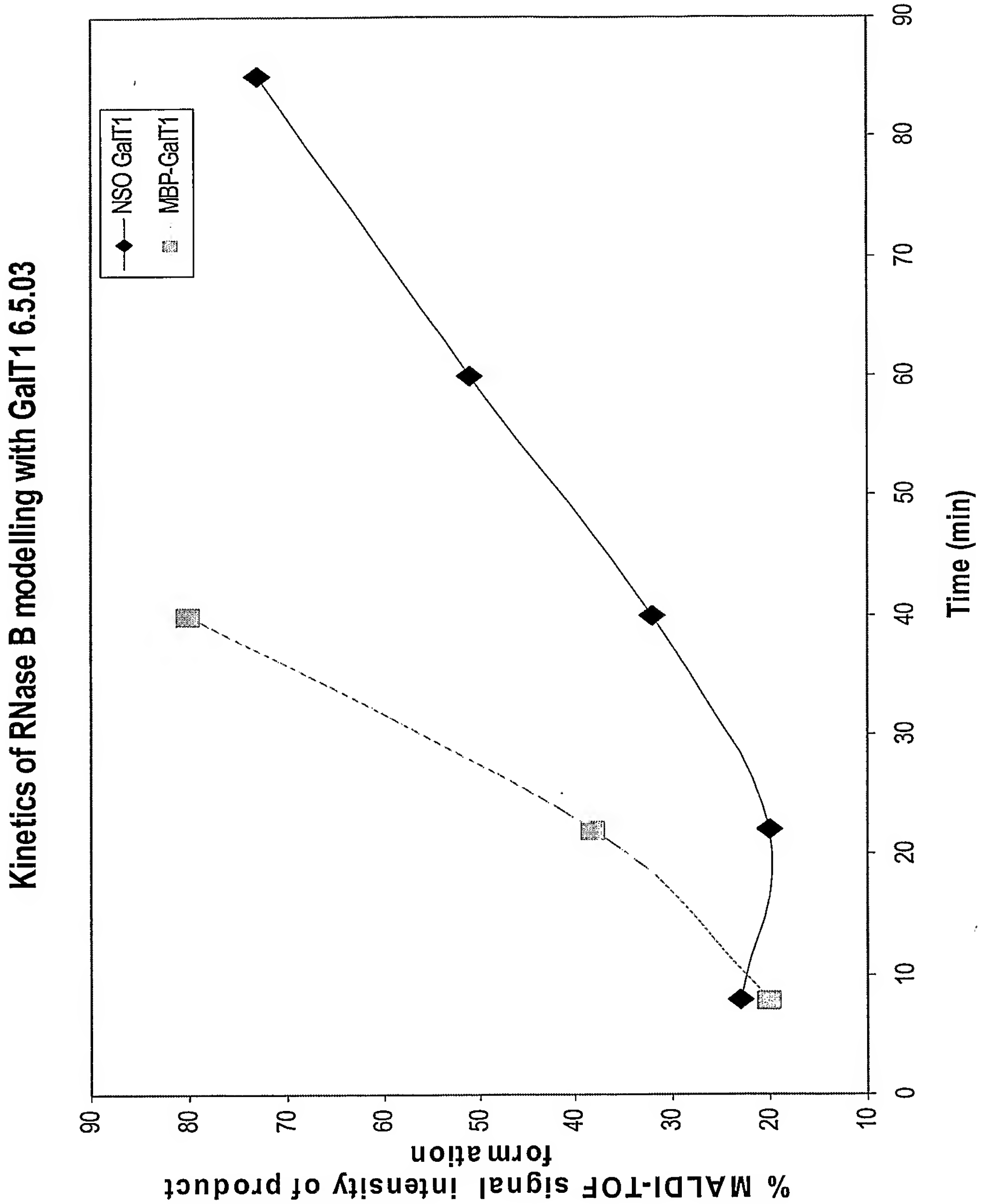
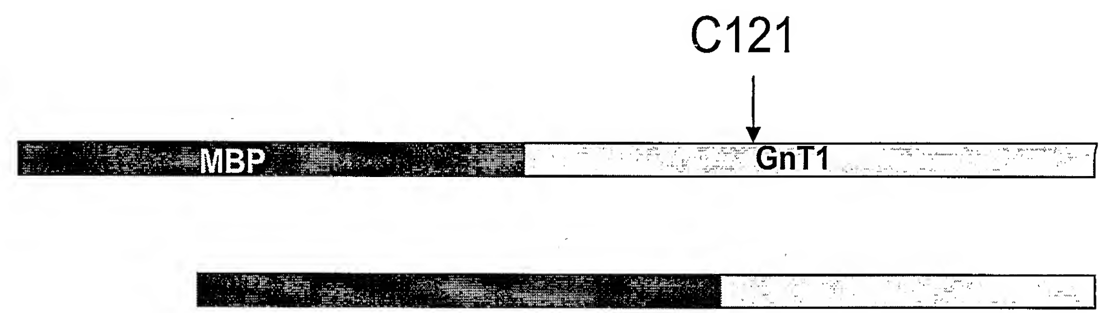

Kinetics of RNase B modelling with GalT1 6.5.03
% MALDI-TOF signal intensity of product formation
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
Time (min)
NSO GalT1
MBP-GalT1

Figure 34

GnT1 constructs

MBP-GnT1(Δ35)

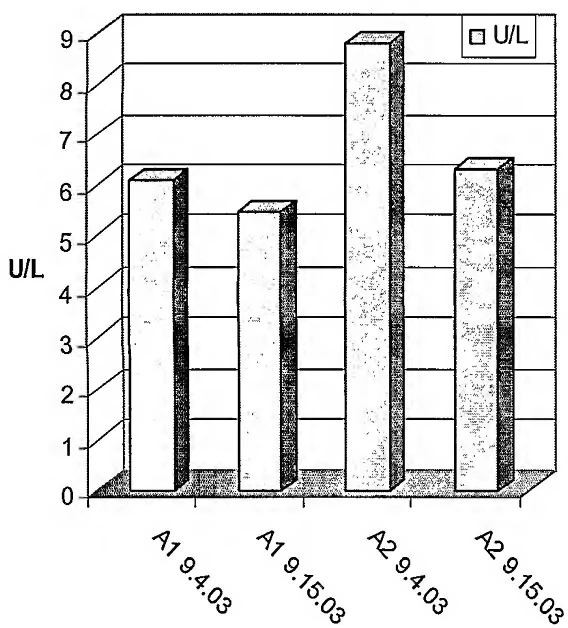
MBP-GnT1(Δ103)




```
1   mlkkqsaglv lwgailfvaw nallllffwt rpapgrppsv saldgdpasl trevirlaqd
61  aevelerqrg llqqigdals sqrgrvptaa ppaqprvpvt papavipilv iacdrstvrr
121 cldkllhyrp saelfpiivs qdcgheetaq aiasygsavt hirqpdlssi avppdhrkfq
181 gyykiarhyr walgqvfrqf rfpaavvved dlevapdffe yfratypllk adpslwcvsa
241 wndngkeqmv dasrpellyr tdffpglgwl llaelwaele pkwpkafwdd wmrrpeqrqg
301 racirpeisr tmtfgrkgvs hgqffdqhlk fiklnqqfvh ftqldlsylq reaydrdfla
361 rvygapqlqv ekvrtndrke lgevrvqytg rdsfkafaka lgvmddlksg vpragyrgiv
421 tfqfrgrrvh lappptwegy dpswn
```

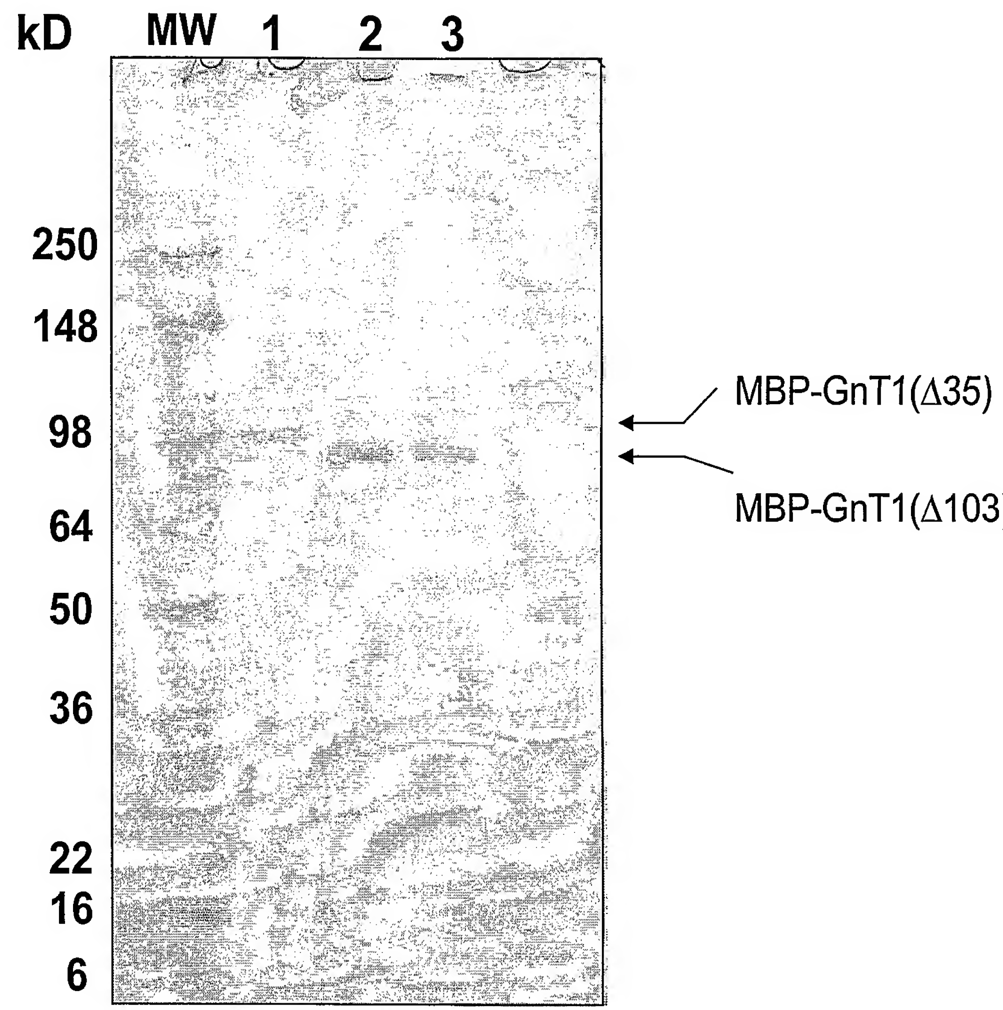
Figure 35

Activities of
Two different batches (A1 and A2) of
Refolded* MBP-GnT1(Δ35) C121A
at different time points



SS 636-127

Comparison of refolded Δ103 and Δ35 truncations of MBP-GnT1



1- MBP-GnT1(Δ35) C121A
2- MBP-GnT1(Δ103) R120A + C121H
3- MBP-GnT1(Δ103) C121A

SS 636-49

Figure 36

Figure 37

```
  1 mapmrkkstl klltllvlfi fltsfflnys htvvttawfp kqmvielsen fkklmkypyr
 61 pctctrciee qrvsawfder fnrsmqpllt aknahleedt ykwwlrlqre kqpnnlndti
121 relfqvvpgn vdpllekrlv scrrcavvgn sgnlkesyyg pqidshdfvl rmnkaptegf
181 eadvgsktth hfvypesfre laqevsmilv pfkttdlewv isatttgtis htyvpvpaki
241 kvkkekiliy hpafikyvfd rwlqghgryp stgilsvifs lhicdevdly gfgadskgnw
301 hhywennpsa gafrktgvhd gdfesnvtti lasinkirif kgr
```

Figure 38

A. Human ST6GalNAcI
MRSCLWRCRHLSQGVQWSLLLAVLVFFLFALPSFIKEPQTKPSRHQRTENIKERSLQS
LAKPKSQAPTRARRTTIYAEPVPENNALNTQTQPKAHTTGDRGKEANQAPPEEQDK
VPHTAQRAAWKSPEKEKTMVNTLSPRGQDAGMASGRTEAQSWKSQDTKTTQGNG
GQTRKLTASRTVSEKHQGKAATTAKTLIPKSQHRMLAPTGAVSTRTRQKGVTTAVIP
PKEKKPQATPPPAPFQSPTTQRNQRLKAANFKSEPRWDFEEKYSFEIGGLQTTCPDSV
KIKASKSLWLQKLFLPNLTLFLDSRHFNQSEWDRLEHFAPPFGFMELNYSLVQKVVT
RFPPVPQQQLLLASLPAGSLRCITCAVVGNGGILNNSHMGQEIDSHDYVFRLSGALIK
GYEQDVGTRTSFYGFTAFSLTQSLLILGNRGFKNVPLGKDVRYLHFLEGTRDYEWLE
ALLMNQTVMSKNLFWFRHRPQEAFREALHMDRYLLLHPDFLRYMKNRFLRSKTLD
GAHWRIYRPTTGALLLLTALQLCDQVSAYGFITEGHERFSDHYYDTSWKRLIFYINH
DFKLEREVWKRLHDEGIIRLYQRPGPGTAKAKN

B. Chicken ST6GalNAcI
MGFLIRRLPKDSRIFRWLLILTVFSFIITSFSALFGMEKSIFRQLKIYQSIAHMLQVDTQ
DQQGSNYSANGRISKVGLERDIAWLELNTAVSTPSGEGKEEQKKTVKPVAKVEEAK
EKVTVKPFPEVMGITNTTASTASVVERTKEKTTARPVPGVGEADGKRTTIALPSMKE
DKEKATVKPSFGMKVAHANSTSKDKPKAEEPPASVKAIRPVTQAATVTEKKKLRAA
DFKTEPQWDFDDEYILDSSSPVSTCSESVRAKAAKSDWLRDLFLPNITLFIDKSYFNV
SEWDRLEHFAPPYGFMELNYSLVEEVMSRLPPNPHQQLLLANSSSNVSTCISCAVVG
NGGILNNSGMGQEIDSHDYVFRVSGAVIKGYEKDVGTKTSFYGFTAYSLVSSLQNLG
HKGFKKIPQGKHIRYIHFLEAVRDYEWLKALLLDKDIRKGFLNYYGRRPRERFDEDF
TMNKYLVAHPDFLRYLKNRFLKSKNLQKPYWRLYRPTTGALLLLTALHLCDRVSAY
GYITEGHQKYSDHYYDKEWKRLVFYVNHDFNLEKQVWKRLHDENIMKLYQRS

C. Mouse ST6GalNAcI protein beginning at residue 32 of the native mouse protein
DPRAKDSRCQFIWKNDASAQENQQKAEPQVPIMTLSPRVHNKESTSVSSKDLKKQER
EAVQGEQAEGKEKRKLETIRPAPENPQSKAEPAAKTPVSEHLDKLPRTPGALSTRKTP
MATGAVPAKKKVVQATKSPASSPHPTTRRRQRLKASEFKSEPRWDFEEEYSLDMSSL
QTNCSASVKIKASKSPWLQNIFLPNITLFLDSGRFTQSEWNRLEHFAPPFGFMELNQSL
VQKVVTRFPPVRQQQLLLASLPTGYSKCITCAVVGNGGILNDSRVGREIDSHDYVFR
LSGAVIKGYEQDVGTRTSFYGFTAFSLTQSILILGRRGFQHVPLGKDVRYLHFLEGTR
NYEWLEAMFLNQTLAKTHLSWFRHRPQEAFRNALDLDRYLLLHPDFLRYMKNRFL
RSKTLDTAHWRIYRPTTGALLLLTALHLCDKVSAYGFITEGHQRFSDHYYDTSWKRL
IFYINHDFRLERMVWKRLHDEGIIWLYQRPQSDKAKN

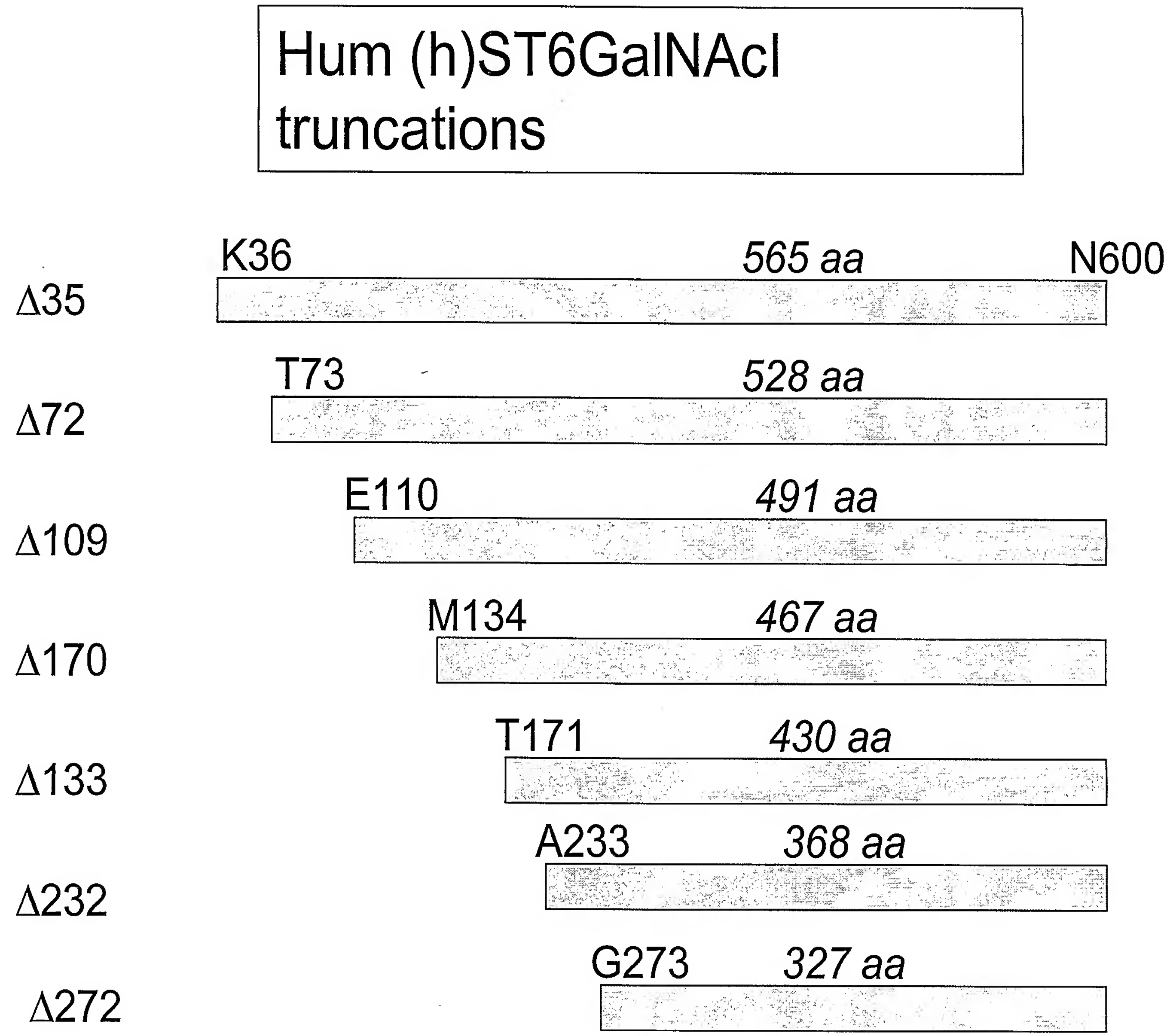

Hum (h)ST6GalNAcI
truncations
Δ35
K36
565 aa
N600
Δ72
T73
528 aa
Δ109
E110
491 aa
Δ170
M134
467 aa
Δ133
T171
430 aa
Δ232
A233
368 aa
Δ272
G273
327 aa

Figure 39

MBP-hST6GalNAcI constructs

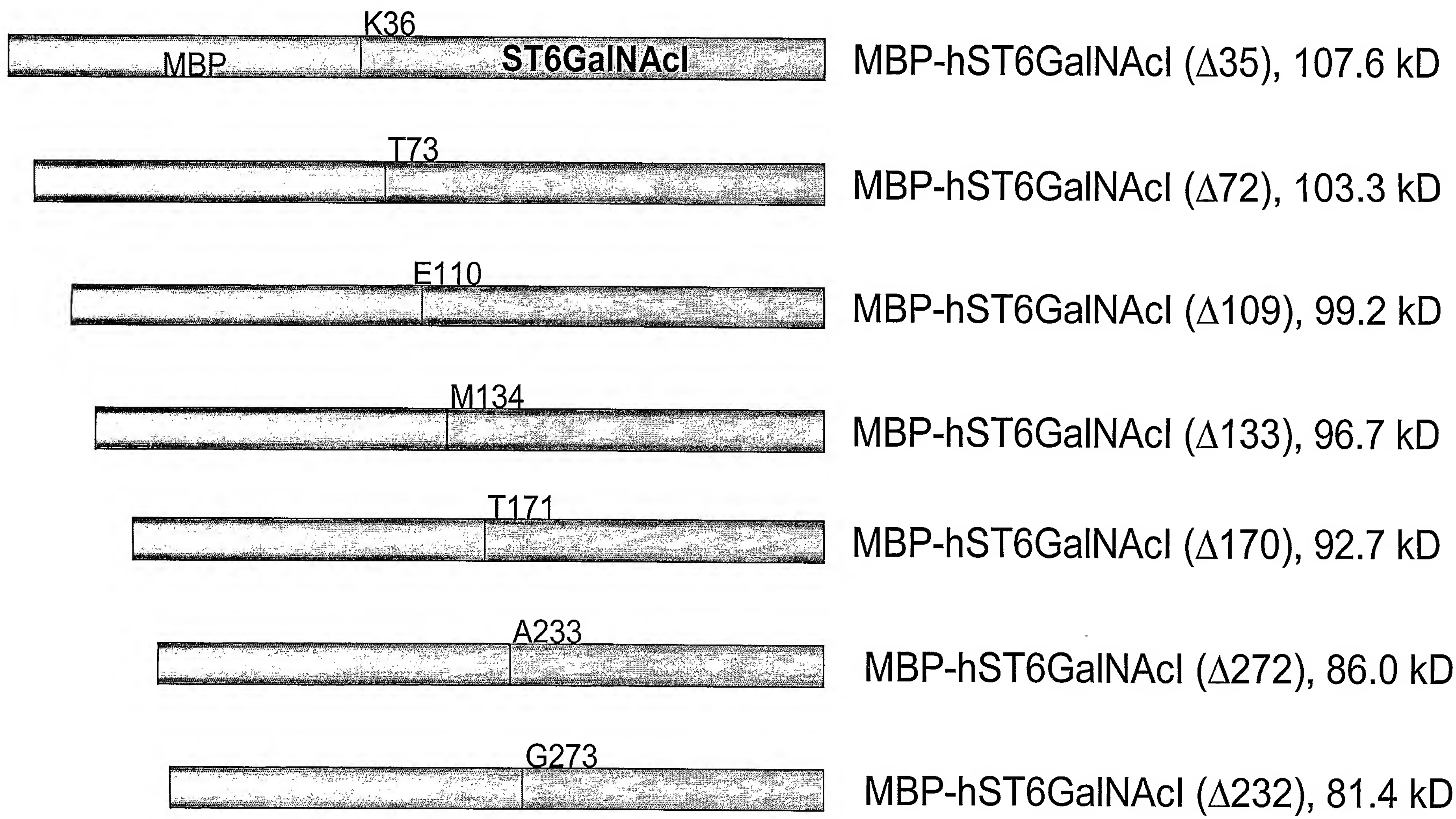


Figure 40

Figure 41

MASKSWLNFLTFLCGSAIGFLLCSQLFSILLGEKVDTQPNVLHNDPHARHSDDNGQN
HLEGQMNFNADSSQHKDENTDIAENLYQKVRILCWVMTGPQNLEKKAKHVKATW
AQRCNKVLFMSSEENKDFPAVGLKTKEGRDQLYWKTIKAFQYVHEHYLEDADWFL
KADDDTYVILDNLRWLLSKYDPEEPIYFGRRFKPYVKQGYMSGGAGYVLSKEALKR
FVDAFKTDKCTHSSSIEDLALGRCMEIMNVEAGDSRDTIGKETFHPFVPEHHLIKGYL
PRTFWYWNYNYYPPVEGPGCCSDLAVSFHYVDSTTMYELEYLVYHLRPYGYLYRY
QPTLPERILKEISQANKNEDTKVKLGNP

```
Sequence        Size        Range        Mode
SC1GALT1.AMI     342          1-      342 Normal
SPTS122.AMI      342          1-      342 Normal
                        10         20         30         40         50
SC1GALT1.AMI       1  EFMPYDGHRH GDVNDAHHSH DMMEMSGPEQ DVGGHEHVHE NSTIAERLYS   50
SPTS122.AMI        1  EFMPYDGHRH GDVNDAHHSH DMMEMSGPEQ DVGGHEHVHE NSTIAERLYS   50
                        60         70         80         90        100
SC1GALT1.AMI      51  EVRVLCWIMT NPSNHQKKAR HVKRTWGKRC NKLIFMSSAK DDELDAVALP  100
SPTS122.AMI       51  EVRVLCWIMT NPSNHQKKAR HVKRTWGKRC NKLIFMSSAK DDELDAVALP  100
                       110        120        130        140        150
SC1GALT1.AMI     101  VGEGRNNLWG KTKEAYKYIY EHHINDADWF LKADDDTYTI VENMRYMLYP  150
SPTS122.AMI      101  VGEGRNNLWG KTKEAYKYIY EHHINDADWF LKADDDTYTI VENMRYMLYP  150
                       160        170        180        190        200
SC1GALT1.AMI     151  YSPETPVYFG CKFKPYVKQG YMSGGAGYVL SREAVRRFVV EALPNPKLCK  200
SPTS122.AMI      151  YSPETPVYFG CKFKPYVKQG YMSGGAGYVL SREAVRRFVV EALPNPKLCK  200
                       210        220        230        240        250
SC1GALT1.AMI     201  SDNSGAEDVE IGKCLQNVNV LAGDSRDSNG RGRFFPFVPE HHLIPSHTDK  250
SPTS122.AMI      201  SDNSGAEDVE IGKCLQNVNV LAGDSRDSNG RGRFFPFVPE HHLIPSHTDK  250
                       260        270        280        290        300
SC1GALT1.AMI     251  KFWYWQYIFY KTDEGLDCCS DNAISFHYVS PNQMYVLDYL IYHLRPYGII  300
SPTS122.AMI      251  KFWYWQYIFY KTDEGLDCCS DNAISFHYVS PNQMYVLDYL IYHLRPYGII  300
                       310        320        330        340        350
SC1GALT1.AMI     301  NTPDALPNKL AVGELMPEIK EQATESTSDG VSKRSAETKT Q*........  350
SPTS122.AMI      301  NTPDALPNKL AVGELMPEIK EQATESTSDG VSKRSTETKT Q*........  350
```

Figure 43

A. *Yersinia* MBP

MKIEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTIEH PDKLEEKFPQ
VAATGDGPDI IFWAHDRFGG YAQSGLLAEL TPSKAFQEKL FPFTWDAVRF
NGKLIGYPVA VEALSLIYNK DLVKEAPKTW EEIPALDKTL RANGKSAIMW
NLQEPYFTWP VIAADGGYAF KFENGVYDAK NVGVNNAGAQ AGLQFIVDLV
KNKHINADTD YSIAEAAFNK GETAMTINGP WAWSNIDKSK INYGVTLLPT
FHGQPSKPFV GVLTAGINAA SPNKELATEF LENYLITDQG LAEVNKDKPL
GAVALKSFQE QLAKDPRIAA TMDNATNGEI MPNIPQMAAF WYATRSAVLN
AITGRQTVEA ALNDAATRIT K

B. *E. coli* MBP

MKIEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ
VAATGDGPDI IFWAHDRFGG YAQSGLLAEI TPDKAFQDKL YPFTWDAVRY
NGKLIAYPIA VEALSLIYNK DLLPNPPKTW EEIPALDKEL KAKGKSALMF
NLQEPYFTWP LIAADGGYAF KYENGKYDIK DVGVDNAGAK AGLTFLVDLI
KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK VNYGVTVLPT
FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL
GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN
AASGRQTVDE ALKDAQTNS

C. *Pyrococcus furiosus* MBP

MKIEEGKVVI WHAMQPNELE VFQSLAEEYM ALSPEVEIVF EQKPNLEDAL
KAAIPTGQGP DLFIWAHDWI GKFAEAGLLE PIDEYVTEDL LNEFAPMAQD
AMQYKGHYYA LPFAAETVAI IYNKEMVSEP PKTFDEMKAI MEKYYDPANE
KYGIAWPINA YFISAIAQAF GGYYFDDKTE QPGLDKPETI EGFKFFFTEI
WPYMAPTGDY NTQQSIFLEG RAPMMVNGPW SINDVKKAGI NFGVVPLPPI
IKDGKEYWPR PYGGVKLIYF AAGIKNKDAA WKFAKWLTTS EESIKTLALE
LGYIPVLTKV LDDPEIKNDP VIYGFGQAVQ HAYLMPKSPK MSAVWGGVDG
AINEILQDPQ NADIEGILKK YQQEILNNMQ G

D. *Thermococcus litoralis* MBP

MKIEEGKIVF AVGGAPNEIE YWKGVIAEFE KKYPGVTVEL KRQATDTEQR
RLDLVNALRG KSSDPDVFLM DVAWLGQFIA SGWLEPLDDY VQKDNYDLSV
FFQSVINLAD KQGGKLYALP VYIDAGLLYY RKDLLEKYGY SKPPETWQEL
VEMAQKIQSG ERETNPNFWG FVWQGKQYEG LVCDFVEYVY SNGGSLGEFK
DGKWVPTLNK PENVEALQFM VDLIHKYKIS PPNTYTEMTE EPVRLMFQQG
NAAFERNWPY AWGLHNADDS PVKGKVGVAP LPHFPGHKSA ATLGGWHIGI
SKYSDNKALA WEFVKFVESY SVQKGFAMNL GWNPGRVDVY DDPAVVSKSP
HLKELRAVFE NAVPRPIVPY YPQLSEIIQK YVNSALAGKI SPQEALDKAQ
KEAEELVKQY SK

E. *Thermatoga maritime* MBP

MKIEQTKLTI WSSEKQVDIL QKLGEEFKAK YGIPVEVQYV DFGSIKSKFL
TAAPQGQGAD IIVGAHDWVG ELAVNGLIEP IPNFSDLKNF YDTALKAFSY
GGKLYGVPYA MEAVALIYNK DYVDSVPKTM DELIEKAKQI DEEYGGEVRG
FIYDVANFYF SAPFILGYGG YVFKETPQGL DVTDIGLANE GAVKGAKLIK
RMIDEGVLTP GDNYGTMDSM FKEGLAAMII NGLWAIKSYK DAGINYGVAP
IPELEPGVPA KPFVGVQGFM INAKSPNKVI AMEFLTNFIA RKETMYKIYL

ADPRLPARKD VLELVKDNPD VVAFTQSASM GTPMPNVPEM APVWSAMGDA
LSIIINGQAS VEDALKEAVD KIKAQIEK

F. *Vibrio cholerae* MBP
MKIEEGQLTI WINGDKGYNG LAEVGKKFEA DTGIKVTVAH PDALQDKFPQ
TAATGDGPDI VFWAHDRFGG YAEAGLLVEI KPSAKIQEGI VDFAWDAVKY
NGKIIGYPIA VESLSLIYNK DLVPNPPKSW EEVAELDAKL KKEGKSAIMW
NLKEPYFTWP LMAADGGYAF KYGVDGYDVK DAGINNKGVK DAMNFVKGLV
DKGVISPDMD YSVSESAFNQ GNTAMTINGP WSWGNIEKSG INYGVTTLPK
FNGQASKPFV GVLTAGISTA SPNKDLAVEF IENYLLTNDG LRMVNNDKPL
GAVALNSFQR ELDADARIAA TMDNAMNGEI MPNIPQMNAF WSSAKNAIIN
IVDGRQTVDA ALADAEKQMT KP

Fig 43 2/2

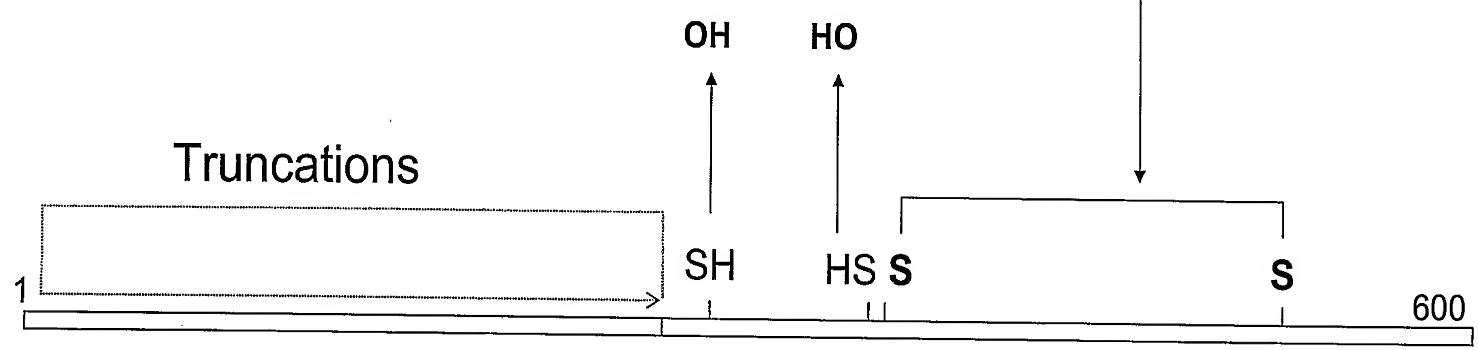
Figure 44

```
                        1                                                 50
HSGALNAT1.pep    (1)    MRKFAYCKVVLATSLIWVLLDMFLLLYFS----------------ECNKC
HSGALNAT2.pep    (1)    MRRRS--RMLLCFAFLWVLGIAYYMYSGGGSALAGGAGGGAGRKEDWNEI
    Consensus    (1)    MRK A   KMLL  A IWVL   F L                   D N
                        51                                               100
HSGALNAT1.pep   (35)    DEKKERGLPAGDVLEPVQKPHEGP-G-----EMGKPVVIPKEDQEKMKEM
HSGALNAT2.pep   (49)    DPIKKKDLHHSNGEEKAQSMETLPPGKVRWPDFNQEAYVGGTMVRSGQDP
    Consensus   (51)    D  K K L        E Q     P G     D      I         D
                        101                                              150
HSGALNAT1.pep   (79)    FKINQFNLMASEMTALNRSLPDVRLEGCKTKVYPDNLPTTSVVIVFHNEA
HSGALNAT2.pep   (99)    YARNKFNQVESDKLRMDRAIPDTRHDQCQRKQWRVDLPATSVVITFHNEA
    Consensus  (101)    F  N FN  M SD  I L RAIPD R D C  K W   LP TSVVI FHNEA
                        151                                              200
HSGALNAT1.pep  (129)    WSTLLRTVHSVINRSPRHMIEEIVLVDDASERDFLKRPLESYVKKLKVPV
HSGALNAT2.pep  (149)    RSALLRTVVSVLKKSPPHLIKEIILVDDYSN-----DPEDGALLGKIEKV
    Consensus  (151)     S LLRTV SVI KSP HLI EIILVDD S         P D   L      V
                        201                                              250
HSGALNAT1.pep  (179)    HVIRMEQRSGLIRARLKGAAVSKGQVITFLDAHCECTVGWLEPLLARIKH
HSGALNAT2.pep  (194)    RVLRNDRREGLMRSRVRGADAAQAKVLTFLDSHCECNEHWLEPLLERVAE
    Consensus  (201)     VIR D R GLIRARLKGA  A A VITFLDAHCEC   WLEPLL RI
                        251                                              300
HSGALNAT1.pep  (229)    DRRTVVCPIIDVISDDTFEYMAGSDMTYGGFNWKLNFRWYPVPQREMDRR
HSGALNAT2.pep  (244)    DRTRVVSPIIDVINMDNFQYVGASADLKGGFDWNLVFKWDYMTPEQRRSR
    Consensus  (251)    DR  VV PIIDVI  D F YMAAS    GGF W L FKW  M         R
                        301                                              350
HSGALNAT1.pep  (279)    KGDRTLPVRTPTMAGGLFSIDRDYFQEIGTYDAGMDIWGGENLEISFRIW
HSGALNAT2.pep  (294)    QGNPVAPIKTPMIAGGLFVMDKFYFEELGKYDMMMDVWGGENLEISFRVW
    Consensus  (301)     G     PIKTP IAGGLF IDK YF EIG YD  MDIWGGENLEISFRIW
                        351                                              400
HSGALNAT1.pep  (329)    QCGGTLEIVTCSHVGHVFRKATPYTFPGGTGQIINKNNRRLAEVWMDEFK
HSGALNAT2.pep  (344)    QCGGSLEIIPCSRVGHVFRKQHPYTFPGGSGTVFARNTRRAAEVWMDEYK
    Consensus  (351)    QCGGSLEII CS VGHVFRK  PYTFPGGSG I  KN RR AEVWMDEFK
                        401                                              450
HSGALNAT1.pep  (379)    NFFYIISPGVTKVDYGDISSRVGLRHKLQCKPFSWYLENIYPDSQIPRHY
HSGALNAT2.pep  (394)    NFYYAAVPSARNVPYGNIQSRLELRKKLSCKPFKWYLENVYPELRVPDHQ
    Consensus  (401)    NFFY   P    V YG I SRL LR KL CKPF WYLENIYPD  IP H
                        451                                              500
HSGALNAT1.pep  (429)    FSLGEIRNVETNQCLDNMARKENEKVGIFNCHGMGGNQVFSYTANKEIRT
HSGALNAT2.pep  (444)    DIAFGALQQGTN-CLDTLGHFADGVVGVYECHNAGGNQEWALTKEKSVKH
    Consensus  (451)            N  TN CLD LA       VGIF CH  GGNQ FA T  K IK
                        501                                              550
HSGALNAT1.pep  (479)    DDLCLDVSKLN--GPVTMLKCHHLKGNQLWEYDPVKLTLQHVNSNQCLDK
HSGALNAT2.pep  (493)    MDLCLTVVDRAPGSLIKLQGCRENDSRQKWEQIEGNSKLRHVGSNLCLDS
    Consensus  (501)     DLCL V          I L  C       Q WE        L HV SN CLD
                        551                        584
HSGALNAT1.pep  (527)    ATEEDSQVPSIRDCNGSRSQQWLLRNVTLPEIF-
HSGALNAT2.pep  (543)    RTAK-SGGLSVEVCGPALSQQWKFTLNLQQ----
    Consensus  (551)     T   S   SI  C  A SQQW
```

CYSTEINE ENGINEERING IN hum ST6GalNAcI

Cys 280 → Ser 280 (*Aat* II)		Restriction sites created
Cys 362 → Ser 362 (*Bgl* II)		
Cys 362 → Thr 362 (*Acc* III)		

Cys 365
Cys 533
Disulfide bridge



Single mutants: C280S, C362S, C362T
Double mutants: [C280S + C362S] , [C280S + C362T]

Figure 45